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SYMPOSIUM ON CELLULAR AND
MOLECULAR ASPECTS OF
ANAESTHESIA

Editors: C. PRYS ROBERTS
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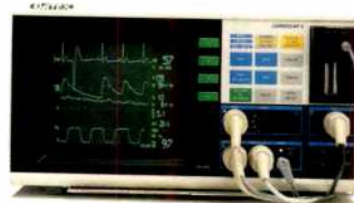
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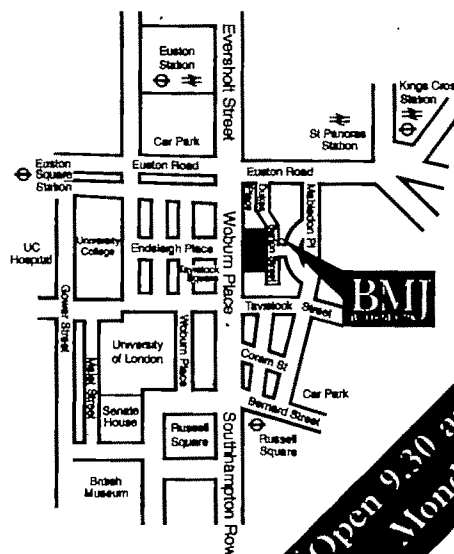
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BRITISH JOURNAL OF ANAESTHESIA

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EDITORIAL

RECEPTORS AND TRANSMEMBRANE SIGNALLING: CELLULAR AND MOLECULAR ASPECTS OF ANAESTHESIA

This postgraduate issue of *British Journal of Anaesthesia* is based on a two-day symposium (11–12 December, 1992), arranged by the Royal College of Anaesthetists in association with *British Journal of Anaesthesia*. It was one of the most successful gatherings of its kind, as it brought together clinical anaesthetists and basic scientists from the United Kingdom, Germany and the U.S.A. The symposium provided the opportunity for these multidisciplinary research workers to interact with each other at a closed workshop, and then present their current "state of the art" work at an open meeting. The resulting review papers, which were revised after the meeting, provide the opportunity for a considered view of the subject to be brought to the attention of the speciality as a whole.

There are a number of unifying concepts that underly the different presentations. First, all would recognize that clinical anaesthesia is staggeringly successful. The certainty of action of a neuronal depressant has come to be taken for granted. However, the more that is known about the multiplicity of underlying perturbations, the more surprising is the overall phenomenon. Anaesthetics are one of the few groups of drugs that are used clinically without real understanding of their underlying mechanisms. In the 146 years since the first fully effective demonstration of general anaesthesia in man, the many theories of how anaesthetics work have originated primarily from the basic sciences. Although the fields of interest of anaesthetists have developed and expanded widely over the last 25 years, it is only recently that there has been a significant contribution to research into the basic mechanisms of anaesthesia from those engaged in clinical practice. One of the problems is that the clinical anaesthetist is more concerned with the visceral and physiological responses to the noxious stimuli, whereas consciousness appears to be more closely associated with somatic responses. There have been many studies in the latter area but, as yet, they have not come to a definitive conclusion.

Some of the work in the area of anaesthesia mechanisms has stemmed from basic advances in cell electrophysiology. The work has been stimulated by the application of molecular biology to neurophysiology. As the understanding of cell functions

moves to a sounder basis, it is appropriate to study the application of anaesthetics. However, there are still a series of important questions that need to be asked, specifically, what do we want to know and why? A number of the review articles in this issue have therefore included a statement about what anaesthetics do not do, as well as what they do. The next question to decide is the future direction of the specific studies of anaesthetics. Some investigators have concentrated on the ion channels themselves, whereas others have emphasized the ion channel complex. The distinction between a pure synaptic voltage-modulated channel and conventional ligand-gated channel has been blurred by the discovery of intermediate forms of ion channels. Everybody is in agreement that the overall cellular effects are far more complicated than they thought 25 years ago.

Two distinctive attitudes are apparent in regard to the differences between anaesthetics in clinical use. The practising anaesthetist may view them as substances which are readily distinguishable by their individual clinical effects. However, the symposium brought together clinical anaesthetists with molecular biologists, pharmacologists and neurophysiologists. Within the latter's research fields, anaesthesia is recognized as being produced by a variety of chemical compounds and the apparently unique features of clinical agents relate only to the fact that they have been screened with care and selected because of limited incidental effects. The realization that side actions may be irrelevant to the mechanisms of anaesthesia is stimulating and not accepted by all investigators. Future experimentation may resolve this particular issue.

The editors hope that the review papers which are reported in these pages will help to stimulate continued interest in the mechanisms of action of anaesthetic drugs, and will provide every encouragement to multidisciplinary investigators to continue and expand their efforts.

M. J. HALSEY
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NEURONAL SODIUM AND POTASSIUM CHANNELS: STRUCTURE AND FUNCTION

K. T. WANN

Information transfer over long distances within the central nervous system is achieved because neurones are capable of generating and conducting action potentials. Such action potentials are the all or none signals or messages of the nervous system and depend on the operation of specialized membrane proteins called ion channels. More than 50 years ago, Cole and Curtis [16] showed that the *electrical conductance* of the nerve axon membrane increased during the action potential, suggesting that the movement of ions was involved. In 1952, Hodgkin and Huxley published a classic series of papers [43] describing formally the *ionic basis* of the action potential in terms of sequential membrane permeability changes to Na^+ and K^+ ions. Now, 40 years on, we are on the threshold of describing the *molecular basis* of excitability [10, 14, 15, 27, 54]. Thus the Na^+ and K^+ channels have been elevated from the status of mythical structures in the minds of electrophysiologists to a position where they are in danger of being crystallized. The combination of traditional biophysical and biochemical methods with the powerful techniques of molecular biology has advanced work in this field to the point that the structure of membrane Na^+ and K^+ channels is within reach [14, 15]. This review is intended as an introduction to the properties of neuronal Na^+ and K^+ channels, with some reference to the effects of anaesthetics. It will serve as a background to the material presented in subsequent reviews.

WHAT ARE ION CHANNELS?

Membrane ion channels may be visualized operationally as water filled holes or tunnels, providing an aqueous route for ions, to enable them to traverse the hydrophobic membrane interior. Their function is to translocate or "process" ions and for this reason they are often thought of as transport proteins, analogous to enzymes, their "substrate" being particular ionic species. The transit time for each ion in the channel is short so that the throughput or transport capacity of each channel is large (i.e. 10^6 – 10^8 ions s^{-1}). However, despite the large processing rate for ions, channels can discriminate, for

example between Na^+ and K^+ ions, achieving selectivities of 100:1 (the relative $\text{K}^+:\text{Na}^+$ permeabilities) in the case of neuronal K^+ channels. For selective ion channels, these are named according to their principal permeant ion species, hence Na^+ and K^+ channels. For neuronal Na^+ and K^+ channels, the conductance (or permeability) of the channel is dependent weakly on temperature, suggesting that the energy barriers within the channel are small; ions moving or diffusing through the channel as they would in free solution. Indeed the weak temperature dependence of the ion permeation process of ion channels in general, is a feature which distinguishes this transport mechanism from that of a carrier-mediated process.

In this review, we focus primarily on the subclasses of ion channels which are voltage-gated. For example, voltage-gated Na^+ channels are first opened by membrane depolarization, that is *activated*, but then (after a delay) are also *inactivated* or closed by depolarization. These activation and inactivation reactions are the so-called gating processes and are thought to depend on the operation of different parts of the ion channel machinery (see below). Voltage-gated K^+ channels are opened or activated also by membrane depolarization. Only certain subtypes of K^+ channels, for example the *Drosophila Shaker* A-type, inactivate also after a delay. One of the challenges of present research on ion channels is the prospect of being able to assign different properties, such as high selectivity and voltage-dependent gating (activation and inactivation) to different parts of the channel structure.

In addition to voltage-gated K^+ channels, there are K^+ channels which are (in some cases) voltage-dependent, which can be regulated also by intracellular messengers, such as Ca^{2+} and adenosine triphosphate (ATP).

Na^+ CHANNELS

Subtypes

The prime function of neuronal voltage-gated Na^+ channels is to generate the rapid depolarization of the action potential. When activated, these channels open, permitting the influx of Na^+ ions down a steep electrochemical gradient into the neurone. This generates an inward Na^+ current which can be detected electrophysiologically (fig. 1A, B).

(Br. J. Anaesth. 1993; 71: 2-14)

KEY WORDS

Anaesthetic action. Electrophysiology. General anaesthesia. Local anaesthesia. Sodium and potassium ion channels.

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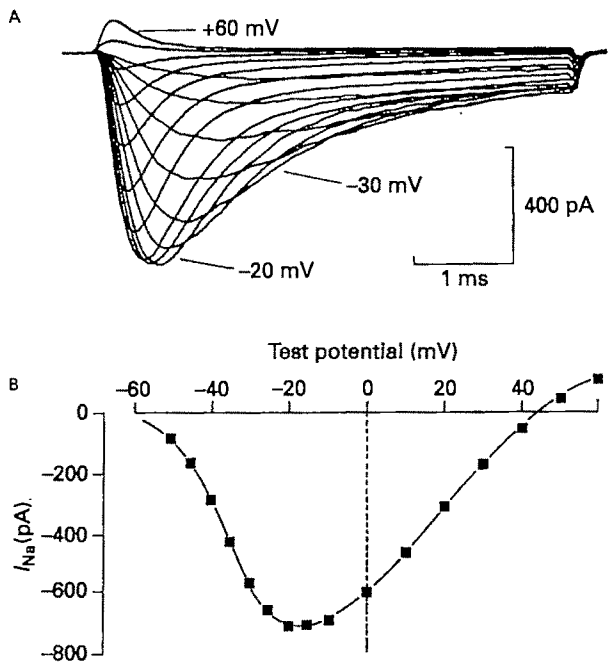


FIG. 1. A: The family of Na⁺ currents elicited in a membrane patch of an oocyte injected with the mRNA specific for rat brain sodium channel II. From a patch holding potential of -100 mV, depolarizing voltage pulses of 5-ms duration were applied to the patch pipette. The applied test potentials were from -50 mV to -10 mV (5-mV increments), -10 mV to +60 mV (10-mV increments). Each record represents the average of eight consecutive responses. B: Peak inward sodium current vs voltage relation for the current records shown in A. The reversal potential = 45 mV (from [86]).

TABLE I. Subunit composition of purified sodium channels

Source	Subunits (kDa)	Core peptide (kDa)
Eel	(α) 280	208
Rat brain	(α) 260	220
	36	23
	33	21
Rat skeletal muscle	(α) 260	
	38	

Although there are strong similarities between Na⁺ channels in different tissues, and indeed in different species, it is clear that subtypes of channels exist (see [9]). This conclusion is based on biochemical evidence, such as the relative sensitivity of the Na⁺ channel types to the neurotoxin, tetrodotoxin, and also electrophysiological estimates of, for example, conductance at the single channel level [91]. There are both electrophysiological data and cloning experiments suggesting that there are Na⁺ channel subtypes in neurones also. In both peripheral nerves and central neurones, there are novel Na⁺ channels which are activated by small depolarizations (“threshold channels”) and slowly inactivate [24, 26, 64, 73, 86]. These Na⁺ channels may be responsible for triggering action potentials and therefore subserve a rather different function to “traditional” voltage-gated Na⁺ channels. How common these channels are is not yet known. Molecular biology also provides strong evidence for Na⁺ channel subtypes in neurones. There are at least

three Na⁺ channel messenger RNA in rat brain from which cDNA and therefore the primary sequences of the respective Na⁺ channels have been obtained [61]. These Na⁺ channel messages showed more than 90 % sequence homology and clearly the individual Na⁺ channels are expressed simultaneously in the brain tissue (although not necessarily in the same neurone).

Density

There are a number of methods of calculating the Na⁺ channel density of nerve membranes. First, this can be estimated from the density of receptor sites for specific toxins, such as tetrodotoxin. Second, this can be deduced from measurement of the total current generated when a population of Na⁺ channel gates open, if the gating charge per channel is known (six equivalent charges per channel). Third, this can be derived by dividing the macroscopic maximal Na⁺ conductance (μm^{-2}) by the single channel conductance (generated by fluctuation analysis or by single channel current measurements). For neurones, estimates of Na⁺ channel density are confined to peripheral nerves: values range from 35–530 μm^{-2} of membrane in unmyelinated nerves to 2000–3000 μm^{-2} of nodal membrane in myelinated systems. The greater the Na⁺ channel density, the more rapid the depolarization rate of the action potential and the greater the conduction velocity. However, there is an optimum number which, when exceeded, results in reduced conduction velocity. At this point the movement of the gating charges (six per channel) as the membrane electric field alters contributes extra capacity current.

Subunit composition

For voltage-gated Na⁺ channels, purification of the protein was possible because naturally occurring high-affinity specific toxins (e.g. tetrodotoxin) were available [2, 36]. The Na⁺ channel from the eel electric organ (a rich source of membrane protein) was found to be a glycoprotein of approximately 1850 amino acids with a molecular weight of 280 kDa (table I). The carbohydrate component of the channel is approximately 20 % w/w (distributed extracellularly) and approximately 50 % of the sugar residues are sialic acids [10]. Na⁺ channels purified from other sources, such as rat brain, possess a similar glycoprotein (the α subunit) and additionally, other smaller glycoprotein subunits (table I). Tetrodotoxin binding studies and Na⁺ flux measurements show that if the α subunit is incorporated into phospholipid bilayers, functional Na⁺ channels can be produced [70]. It appears therefore that the ion channel (aqueous pore and gating processes) resides in the α subunit, the function of the other subunits not yet being known. Patch-clamp studies in oocytes confirm this finding. Injection of oocytes from *Xenopus laevis* with mRNA produced by *in vitro* transcription of the cDNA encoding the sequence for the α subunit of the rat brain Na⁺ channel II (subtypes of Na⁺ channels exist), results in the expression of functional Na⁺ channels with properties similar to the native Na⁺ channel of rat nerve [86].

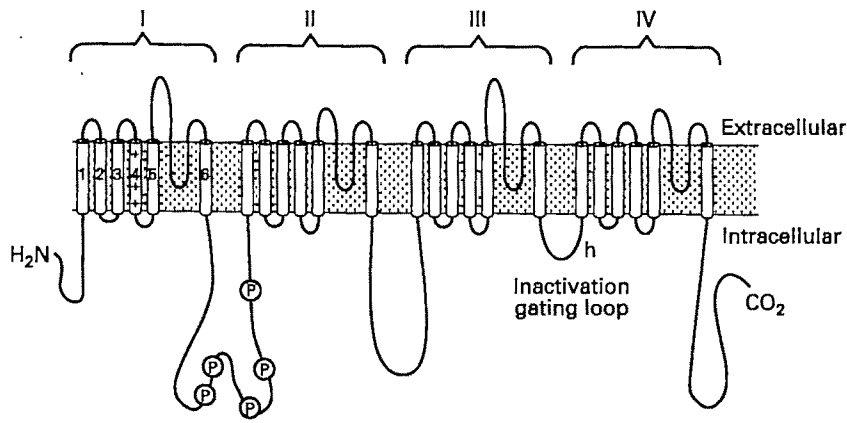


FIG. 2. Proposed transmembrane folding of the amino acid chains of the α subunit of the Na^+ channel. The repeats are labelled I, II, III and IV, the membrane-spanning segments are shown as the cylinders 1–6 and the voltage sensor as the positively charged fourth cylinder. The pore lining links 5 with 6. The cytoplasmic loop (h) is the putative inactivation gate. P shows phosphorylation sites (adapted from [15]).

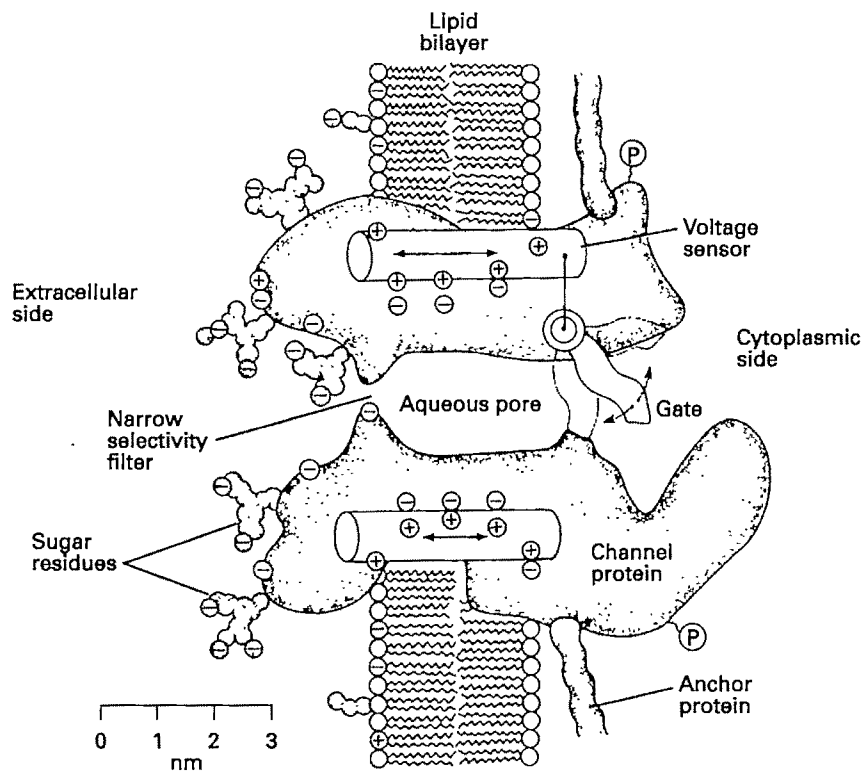


FIG. 3. Schematic diagram of the voltage-gated Na^+ channel protein which spans the membrane. The charged voltage sensor is linked to an internal gate. The pore narrows at the selectivity filter region where selection of ions takes place. Negative charges on the external membrane surface contribute a potential which adds to the electrical field within the membrane (from [41]).

Primary structure of the Na^+ channel

The biochemical identification and purification of the Na^+ channel provided the starting point for cloning of DNA (cDNA) complementary to messenger RNA (mRNA) coding for the Na^+ channel. Analysis of the nucleotide sequence of the cloned cDNA together with knowledge of the genetic code, yielded the entire amino acid sequence of the α subunit protein [62]. It was found that the α subunit consists of four repeated sequences of amino acids (I–IV), and that within each of these repeats there are thought to be six membrane-spanning hydrophobic domains (S1–S6) and connecting intra- and

extracellular loops (see fig. 2). It is believed that the membrane-spanning domains assume an α helical structure. The aqueous pore of the channel is formed in this model by assuming that one or two of the membrane-spanning regions of each repeat are arranged like the staves of a barrel, forming the pore lining. There is evidence from work on the K^+ channel that this function is assumed by the membrane-spanning loop of amino acids between S5 and S6, often referred to as SS1–SS2 (short segments 1 and 2) (see fig. 6).

Several investigations have expanded the arguments so that it is now possible to assign functions to particular regions, or indeed even more impressively,

to specific amino acids. The specific properties of voltage sensitivity, ionic selectivity and inactivation can begin to be described in molecular terms. Also, the target residues for specific toxins, such as tetrodotoxin have been defined. This exciting prospect is made possible by the combined use of site-directed mutagenesis and patch-clamp recording in oocytes injected with mRNA derived from cDNA [35, 85, 86, 88]. One intriguing question is what is the basis of the voltage sensitivity of the Na⁺ channel? One of the membrane-spanning regions, S4, is highly conserved between Na⁺ channels from different species. It is also homologous to the equivalent regions of voltage-gated K⁺ and Ca²⁺ channels. It has therefore been proposed that the S4 region is the voltage sensor of the ion channel, that is this region may detect changes in the electric field within the membrane and respond by producing conformational changes in the channel protein. For membrane depolarization (a less steep electric field) the conformational transition results in Na⁺ channel opening. The simplest voltage sensor to visualize is a structure which is charged and may move under the influence of the electric field. What is unique about the S4 region is that there are positively charged arginine or lysine residues at every third position [62]. These positively charged, but still hydrophobic (and therefore membrane spanning), segments have the appropriate credentials to act as membrane "voltmeters". Supporting this view is the finding that if the positively charged amino acid residues are replaced by neutral or negatively charged residues (by site-directed mutations), the voltage sensitivity of the Na⁺ channel gating is reduced. This is normally deduced from plots of Na⁺ conductance (g_{Na} or m_{∞}^3) vs voltage [85].

Similar experiments indicate that a glutamate residue located in the extracellular loop connecting (in repeat I) the S6 domain with the SS1-SS2 loop (short segments 1 and 2, see figs 2 and 6) is the putative binding site for tetrodotoxin. Neutralizing this acid residue reduces dramatically the sensitivity of the Na⁺ channel to tetrodotoxin [63]. Other mutations indicate that lysine and alanine residues, in repeats III and IV, are critical determinants of the ionic selectivity of the Na⁺ channel. Do these residues form parts of the so-called selectivity filter of the channel structure [39, 40]? An intracellular loop which connects repeats III and IV is likewise thought to be critical for the Na⁺ channel inactivation process. The evidence is that cleavage of this linkage results in a dramatic reduction in the rate of channel inactivation after depolarization [85]. The inactivation machinery has long been known to be accessible to attack by enzymes (e.g. pronase) applied to the cytoplasmic side [6] so that it is not surprising that the inactivation gate resides allegedly on a cytoplasmic loop. In fact, it appears that cytoplasmic domains are important for inactivation of certain voltage-gated K⁺ channels also. This may therefore be an additional uniform feature of channel structure.

Operational model of the Na⁺ channel

It is now possible to imagine an operational model

of the Na⁺ channel protein, which highlights the unique properties of such a structure [41]. Figure 3 shows a schematic version of the channel. Functionally, it is convenient to distinguish between two fundamentally different channel operations: Na⁺ channel *gating* and the Na⁺ *permeation* process. Models of both these processes have been discussed in detail previously [11, 25, 41, 92]. As indicated above, there is now a clearer molecular picture of the voltage sensor which operates the channel gate to open the channel. This sensor responds to changes in the electric field in the membrane and the field profile depends in part on the size of the negative surface potential at the outer and inner surfaces of the membrane. These negative charges may be derived from phosphate groups or sugars, such as sialic acids (see fig. 3). Divalent cations, such as Ca²⁺ and possibly certain anaesthetics (see below), can change the surface potential, modifying the electrical gradient within the membrane. The channel gate is represented as a barrier on the inner side of the channel where it is accessible to attack by chemical reagents.

Voltage-gated Na⁺ channels are either fully open or closed: there are no intermediate conductance levels as is often found in other ion channels. At physiological ion concentrations, the channel is often treated as a one-ion pore, that is only one ion is allowed inside at any time [92]. The principal energy barrier to ion permeation occurs at a region within the channel which is oxygen-lined with an opening of 0.3–0.5 nm [40]. This is the selectivity filter (see fig. 3). Estimates of its size stem from studying what is permeant and what is not. Systematic analysis of the permeability of the Na⁺ channel to different alkali metal cation and organic cations showed that permeability decreased with increasing ionic radius (e.g. Na⁺:K⁺ permeability = 12:1). Some alkali metal cations (Li⁺, Na⁺, K⁺), NH₄⁺ and several organic cations, which can form hydrogen bonds with the channel wall, pass through but no molecules with diameters of 0.6 nm or more are permeant [38–40]. Permeant cations are thought to interact with an acidic group within the channel during transit. Protonation of this group therefore results in reduced Na⁺ channel conductance.

Modification of Na⁺ channel properties

An array of agents modify the properties of neuronal Na⁺ channels. Indeed some early valuable clues to the architecture of the Na⁺ channel were provided by pharmacological studies with a variety of neurotoxins, enzymes and chemical reagents. For example, trimethyloxonium ion, which methylates carboxylic acids, decreases the binding of tetrodotoxin. This provided evidence that these groups are critical for the interaction of the toxin with the Na⁺ channel [81]. Similarly, the finding that inactivation can be removed by intracellularly applied enzymes, such as pronase and trypsin, raised the possibility that residues, such as arginine or lysine, were important for the expression of inactivation. More recently it has been shown that sialic acids may influence Na⁺ channel gating. Treatment of eel

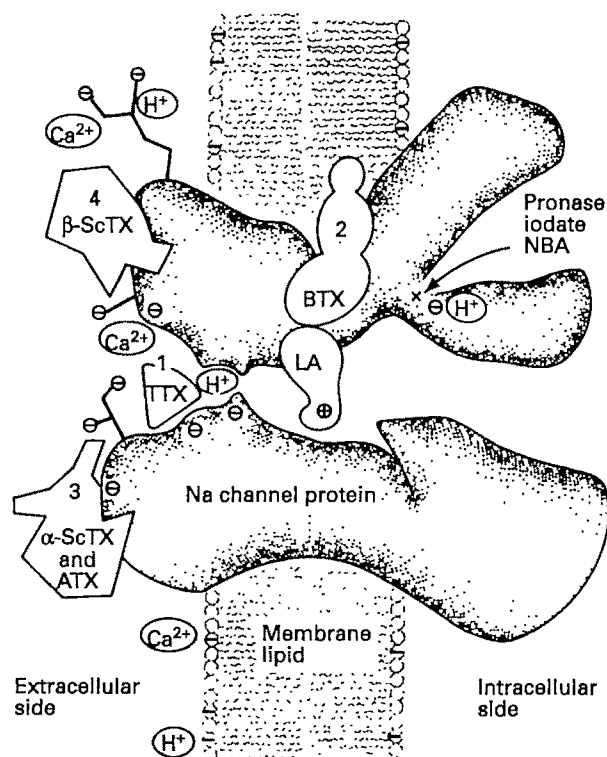


FIG. 4. Receptor sites for toxins, pronase and local anaesthetics on the Na⁺ channel protein. Four separate toxin sites are shown for tetrodotoxin (TTX), batrachotoxin (BTX), scorpion and coelenterate peptide α toxins (α -ScTx and ATX) and scorpion peptide β toxin (β -ScTx). Pronase (together with iodate and N-bromoacetamide (NBA)) acts internally. The local anaesthetics (LA) locate (bind) within the Na⁺ channel (from [41]).

electroplax Na⁺ channels (reconstituted into lipid vesicles) with neuraminidase to remove sialic acids, shifted the activation curve for the Na⁺ channel conductance by more than 30 mV [67].

For Na⁺ channels it is important to distinguish between two classes of drugs with different actions. Toxins such as tetrodotoxin and saxitoxin (found in marine dinoflagellates) act as channel blockers—literally external plugs at the channel mouth. Many other naturally occurring neurotoxins and enzymes, however, modify the *kinetics* of gating. The proposed target sites for some of these agents are shown in figure 4. Clearly there are at least four different target sites for neurotoxins, each of which modifies the channel properties differently (see also [15]). As indicated previously, tetrodotoxin and saxitoxin which carry guanidinium groups (guanidinium ions are permeant) enter the channel and block from the outside. Scorpion and coelenterate peptide and toxins also act from the outside but at a different site to that of tetrodotoxin. These toxins do not block Na⁺ channels, in fact they slow Na⁺ channel inactivation producing persistent inward Na⁺ currents and prolonging the action potential. Scorpion peptide β toxin acts at a third external site to modify the activation of Na⁺ channels. By shifting the voltage dependence of Na⁺ channel activation in an hyperpolarizing direction, this toxin acts as a Na⁺ channel agonist. Nature has provided a wealth of other Na⁺ channel agonists which are lipid soluble toxins and which act at a separate site within the

membrane. Such toxins include the alkaloid veratridine (from the lily family), batrachotoxin (from the Colombian arrow poison frog) and grayanotoxins (from rhododendrons).

The neurotoxins, several enzymes and select chemical reagents have proved to be effective tools to dissect the properties of the neuronal Na⁺ channel providing useful chemical information (in the days before molecular biology) on which to build a molecular picture of the workings of the channel protein.

Anaesthetic interactions

Local anaesthetics block propagating action potentials by “plugging” voltage-gated Na⁺ channels at a site within the channel beyond the cytoplasmic gate. Many other anaesthetic classes (e.g. barbiturates, alcohols, inhalation) also modify Na⁺ channel function [89]. In all cases these agents reduce inward Na⁺ currents. However, it is important to note that this reduction is not always achieved in a uniform way. Several principal effects have been described which may be discussed in the context of the channel model shown in figure 4.

Local anaesthetics are lipid soluble amines which are uncharged at pH > 8.5 and positively charged at pH < 6. The uncharged form is the lipid soluble one. Important clues to the mechanism of action of this class of anaesthetics came from experiments with QX-314 (a quaternary deviate with a permanent positive charge) and GEA-968 (a lignocaine analogue). QX-314 blocks Na⁺ channels only when applied to the inside of the neurone and the drug has to access its binding site through the open cytoplasmic gate [84]. The drug also produces a cumulative block with repeated channel openings [84], that is more anaesthetic becomes bound with each opening of the channel. This is known as use-dependent block and can only be achieved if the anaesthetic does not unbind at rest [19]. Use-dependent block is produced also by the hydrophilic GEA-968 [19] and the more hydrophobic lignocaine. The more frequently the Na⁺ channels are opened, the greater the degree of block (fig. 5). The difference with GEA-968 and lignocaine, however, is that they can exit the closed channel. Hence it is necessary to stimulate at a greater frequency to achieve cumulative block. In this case it appears that there is a hydrophobic route of exit (through adjacent lipid) in addition to a hydrophilic path through the open gate at the channel mouth (fig. 5). This type of model, elaborated by Schwarz, Palade and Hille [76], predicts that the more charged the local anaesthetic agent the closer its action will resemble QX-314 in producing use-dependent block. If the local anaesthetic is neutral, like benzocaine, the hydrophobic pathway dominates the interaction and there is no use-dependent block.

The multiple actions of anaesthetics can best be appreciated by comparing different classes of agents. The work of Haydon and colleagues illustrates this well [32–34, 37]. The hydrocarbons produce clear shifts in the negative direction of the steady state inactivation (h_{∞}) vs voltage curve of the Na⁺ channel of squid axon [32]. These agents also suppress

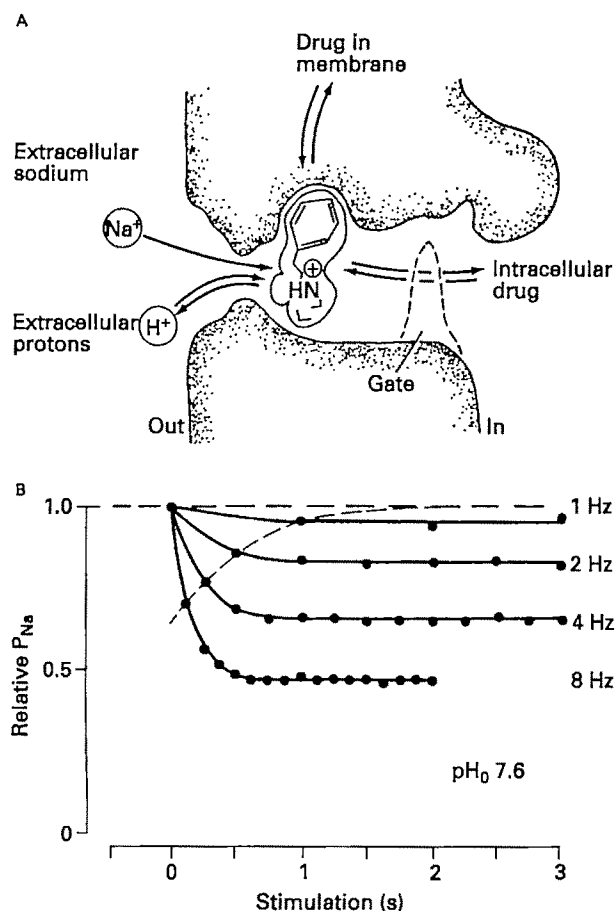


FIG. 5. A: The proposed location of the local anaesthetic binding site within the channel and the possible routes of access to and exit from that site (from [76]). B: Frequency dependent block of the inward Na^+ current in the frog node of Ranvier by lignocaine $0.5 \text{ mmol litre}^{-1}$. The effects of stimulating the nerve at 1, 2, 4 and 8 Hz on the reduction of Na^+ permeability (P_{Na}) by lignocaine are shown. The block achieved by each successive pulse is frequency dependent. Note the data are expressed as a fraction of the initial reduced P_{Na} in the presence of lignocaine and the dotted line is an estimated time course of recovery from the extra block achieved by each first depolarizing pulse (from [42]).

maximum Na^+ conductance ($\bar{g}_{\text{Na}}$). This is explained by proposing that these agents thicken the membrane which is postulated to have two effects. First, the electric field profile would be reduced. This would account for the shift in the h_{∞} curve along the voltage axis. Second, it is suggested that this thickening results in destabilization of the Na^+ channel structure which clearly must span the membrane to remain functional. This might explain the suppression of $\bar{g}_{\text{Na}}$. In comparison, the alcohols and inhalation anaesthetics produced smaller (or negligible) shifts in the h_{∞} curve and smaller (or no) reductions in $\bar{g}_{\text{Na}}$ [33, 34]. The implication is that these agents do not thicken the axon membrane or that another effect offsets any action of this type. In this context it is interesting to note that the alcohols and the inhalation anaesthetics shift the steady state activation *vs* voltage curve (m_{∞}) in a depolarizing direction. This action is ascribed to an unequal effect of some of these agents on the external and internal surface potentials (see fig. 3).

Subtypes

K^+ CHANNELS

In contrast to voltage-gated Na^+ channels, K^+ channels represent a heterogeneous family of membrane proteins [28, 51, 71]. They subserve a number of roles which include setting the resting membrane potential, shaping action potentials, controlling action potential discharge patterns and terminating bursts of activity. Subtypes such as the delayed rectifier, M and A types, are voltage-gated and in some cases regulated also by transmitters (e.g. the M channel is closed by *muscarinic* agonists); others are voltage-dependent and activated by increases in cytoplasmic Ca^{2+} (the maxi- K^+ channel) or activated by increases in cytoplasmic Ca^{2+} , and transmitter regulated (SK channel— Ca^{2+} activated K^+ channel of small conductance); finally, others are regulated by intracellular ATP. Some subtypes (e.g. A and D channels) of K^+ channels activate and then inactivate after depolarization, as in the case of Na^+ channels: others show no inactivation. Detailed descriptions of the properties of these K^+ channel subtypes have been described previously [12, 28, 51, 71]. This review can only hope to provide an introduction to the structure and function of this important ion channel family. Since the equilibrium potential for the K^+ ion is approximately -90 mV under normal physiological conditions, it is clear that activation of any of the K^+ channel subtypes will exert a repolarizing influence or induce hyperpolarization, depending on the voltage at which the channel is activated. In this sense, K^+ channel activation will result in an outward current which will tend to reduce membrane excitability, that is serve as an effective brake on the activity of the neurone.

Density

In the absence of appropriate ligands of high specificity for any K^+ channel, K^+ channel densities cannot be calculated readily by computing the number of binding sites (μm^{-2}) for an attacking molecule. Hence it is necessary to use electrophysiological estimates. As for Na^+ channels, estimates have been made by dividing the macroscopic peak conductance (μm^{-2}) by the single channel conductance. For the delayed rectifier K^+ current of peripheral unmyelinated nerves this yields a density of $20\text{--}30 \mu\text{m}^{-2}$. The density of delayed rectifier channels in the nodal membrane of myelinated nerves is considerably greater ($100\text{--}1000 \mu\text{m}^{-2}$). The density of packing in neuronal membranes of other K^+ channel subtypes is at present unknown.

Primary sequence of the channel

The small abundance of K^+ channel proteins in excitable membranes, coupled with their heterogeneity, has made it impractical to adopt the purification approach utilized so successfully for the Na^+ channel, although progress has been made with particular ligands [60, 68]. Fortunately, a mutation in the K^+ channel gene in *Drosophila* was known and this paved the way for a genetic approach. This *Shaker* mutation results in leg shaking under ether anaesthesia. Electrophysiological studies in *Shaker* mutant larvae showed that transmitter release at

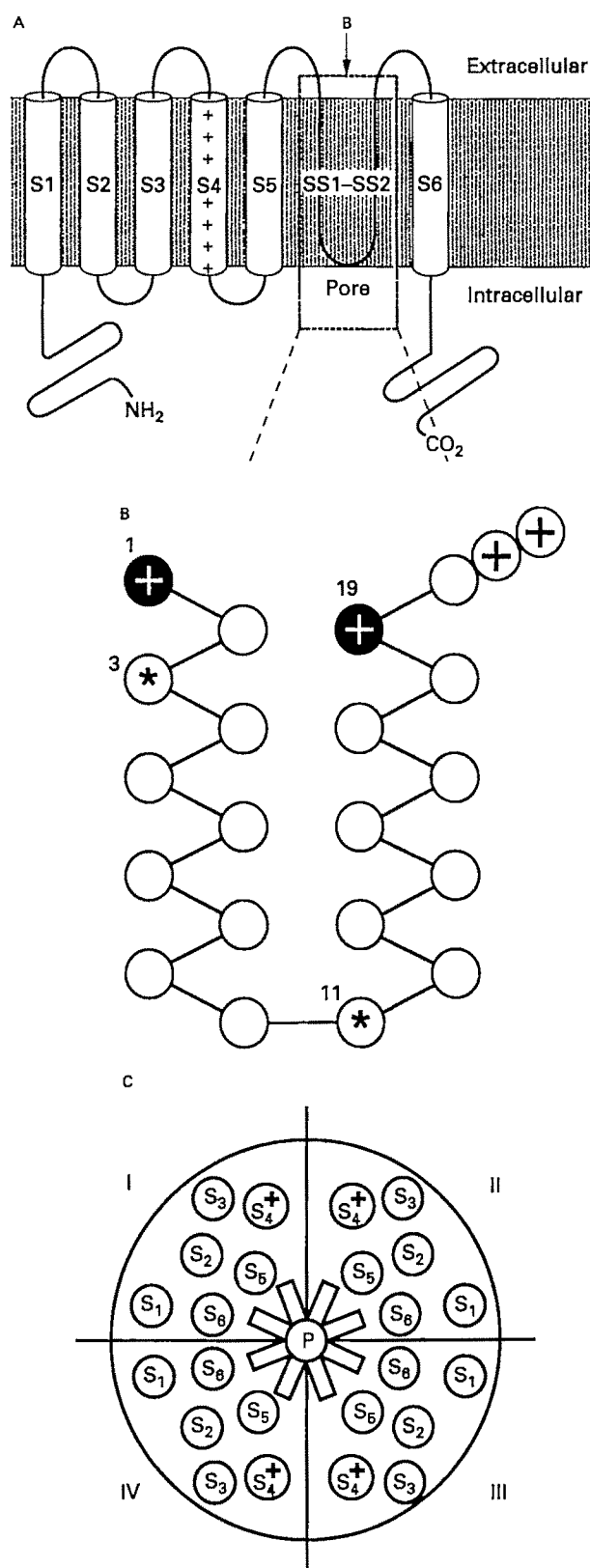


FIG. 6. A: Proposed transmembrane folding of the amino acid chains of the *Shaker* A type K⁺ channel. Note the membrane-spanning segments S1–S6, the positively charged S4 voltage sensor and the SS1–SS2 linkage between S5 and S6 which is the putative pore lining. B: The SS1–SS2 regions consist of 19 amino acids, residues 1, 11 and 19 are the proposed binding sites for tetraethylammonium ion; residues labelled (+) are the target sites for charybdotoxin, mutations at * influence ionic selectivity. C: View looking down the mouth of the pore. The channel is proposed to be tetrameric (I–IV) with the SS1–SS2 loops coming together like the staves of a barrel to form the pore (P).

the neuromuscular junction was increased, thus producing large, prolonged postsynaptic potentials in the larval muscles. Voltage-clamp studies [75] identified the mutation in the A type K⁺ current (a voltage-gated rapidly inactivating K⁺ channel). The chromosomal location of the mutation was mapped and the K⁺ gene was cloned and sequenced. When expressed in oocytes, the clone gives rise to inactivating (A type) K⁺ currents. A number of K⁺ channel genes homologous to the *Shaker* gene have now been cloned in both *Drosophila* and mammalian systems [47, 74, 87]. When these clones are expressed they result in K⁺ channels with different speeds of inactivation. Some clones encode for K⁺ channels which inactivate rapidly (e.g. *Shaker*, *Shal* from *Drosophila*, MBK1 from mouse), others encode for K⁺ channels with slower inactivation (e.g. *Shab* from *Drosophila*). Finally, there are clones which encode for K⁺ channels with no inactivation—a delayed rectifier type K⁺ channel (e.g. *Shaw* from *Drosophila*, RCK1 from rat). The families of K⁺ channel genes (and the confusing nomenclature) are tabulated [47]. Therefore, this suggests one reason at the genomic level for the heterogeneity of K⁺ channels. There are a number of genes which give rise to different channel protein products. Alternatively, post-translational events may be responsible for some of the diversity of K⁺ channels. Post-translational processing appears to confer sensitivity to charybdotoxin. *Shaker* K⁺ channels are sensitive to charybdotoxin when expressed in oocytes but not in the muscle cells of *Drosophila*. The question of K⁺ channel diversity has been discussed in detail elsewhere [47].

The *Shaker* gene for the K⁺ channel encodes for a protein which is equivalent to one of the four subunits of the Na⁺ channel (fig. 6). Functional K⁺ channels are therefore thought to be formed from the co-assembly of four identical (or at least similar) subunits. The evidence that the functional K⁺ channel was tetrameric was derived from experiments where different cDNA were expressed in the same oocytes. Imagine that the protein products of two separate cDNA were different in some way. Perhaps the products (channels) differ in their kinetics of inactivation or sensitivity to charybdotoxin. If the cDNA are now co-expressed then a heterotetrameric K⁺ channel (or hybrid or chimeric K⁺ channel) with properties intermediate between the two extremes was produced [45, 53]. Of course if the subunits of neuronal K⁺ channels are different then such heterotetrameric channels would have properties different to homotetrameric channels. Perhaps this is another reason for K⁺ channel diversity.

What is the arrangement of amino acids within one subunit? This is shown in figure 6. As for the Na⁺ channel, the amino acid chain makes a number of crossings of the membrane. There are six transmembrane-spanning segments (S1–S6), a short loop of amino acids within the membrane between S5 and S6 (known as SS1–SS2, short segments 1 and 2) and significant intracellular domains (the NH₂ and carboxyl termini). The total number of amino acids in the unit is 610.

TABLE II. K^+ channel pharmacology

Channel type	Blocker
Delayed rectifier, M channel, Maxi- K^+ channel	Tetraethylammonium ion
A channel, D channel	Dendrotoxins, aminopyridines
Maxi-channel	Charybdotoxin
Small Ca^{2+} -dependent K^+ channel	Apamin

By analogy with Na^+ channels (see above), the S4 transmembrane segment, with basic residues at every third position, is thought to be the voltage sensor of the K^+ channel. Mutations within this region modify the voltage dependence of activation [52, 65], although substitution of each of the seven positive charges (by glutamine) in turn did not produce an equal reduction in the sensitivity of the channel to voltage [65]. The arguments that the SS1–SS2 loop constitutes the ion pore of the K^+ channel are summarized elsewhere [48, 54]. The pore is thought to be formed from a stretch of approximately 19 amino acids. The evidence for this is derived from analysis of the target residues for charybdotoxin and tetraethylammonium ion and the effects of mutations on ionic selectivity. Site-directed mutagenesis identified two groups of amino acids (separated by a short stretch of amino acids) which are critical for the binding of charybdotoxin [56]. It was proposed that these were present at the mouth of the channel (see fig. 6). Mutations of residues 1 and 19 affect block by external tetraethylammonium ion: a mutation at residue 11 affects block by internal tetraethylammonium ion. Mutations at positions 3 and 11 increased the permeabilities to NH_4^+ and Rb^+ over K^+ [94]. The interpretation of these findings is that the SS1–SS2 region, despite its short length, forms a hairpin turn within the membrane stretching from the outside to the inside channel mouth.

What is the mechanism of K^+ channel inactivation? The *Shaker* K^+ channel family members possess both variable inactivation rates and a different amino acid sequence in the intracellular amino and carboxyl termini. This suggests that these intracellular loops constitute part of the inactivation gating machinery (see Na^+ channels above). The fast inactivation process of the *Shaker* K^+ channel has little voltage dependence: this supports the view that the inactivation gate resides in a domain that does not span the membrane. Site-directed mutagenesis in conjunction with patch-clamp recording in oocytes has pinpointed the inactivation gate [44]. Removal of positively charged residues (lysine and arginine) slowed dramatically the inactivation rate. In an elegant set of experiments, Zagotta, Hoshi and Aldrich showed, in expression studies, that internal application of a synthetic peptide (first 20 amino acids of the NH_2 terminus) to an inside-out membrane patch containing non-inactivating mutant channels restored fast inactivation [95]. The inactivation gate can be visualized physiologically as an open channel blocker [20]. The “receptor” for the inactivation gate is thought to reside with acidic

residues in the S4–S5 loop [46]. The NH_2 terminus gate (20 amino acids) of the channel protein is attached to the cytoplasmic side of the channel (by about 60 amino acids) and somehow “swings in”, binds to the S4–S5 loop and blocks the channel. This is the “ball and chain” model of channel inactivation proposed initially for the Na^+ channel by Armstrong and Bezanilla [5]. One obvious difference between the K^+ channel and the Na^+ channel inactivation processes is that in the case of the former, there are four tethered inactivation gates.

Pharmacology of K^+ channels

Although there are few modulators of K^+ channel function which act with the same specificity as the neurotoxins do for the Na^+ channel, several interesting K^+ channel blockers are available which have been useful both in dissecting macroscopic K^+ currents and in the study of single channels [13, 59, 60, 68]. The more important of these and their targets are shown in table II. Because these ligands block K^+ channels, removing the restraining influence on membrane excitability, these agents (e.g. aminopyridines, dendrotoxin) often have convulsant properties, although there are differences in the type of convulsion produced. The interaction of the tetraethylammonium ion with the delayed rectifier channel has been the most extensively studied and has generated the first “molecular” description of K^+ channel function [3, 4]. Some aspects of this interaction are outlined below. The aminopyridines (e.g. 4-aminopyridine) and dendrotoxin (peptide isolated from the venom of the green mamba, *Dendroaspis angusticeps*) block A and D type K^+ channels which carry transient outward currents [29, 83] in response to depolarization (i.e. they show inactivation). Ca^{2+} -dependent K^+ channels in neurones (as in other tissues) may be distinguished on the basis of their respective single channel conductances. In patch-clamp recordings with symmetrical 140 mmol litre⁻¹ of K^+ solutions, these are approximately 200 pS and 20 pS for the maxi- K^+ channel and the SK K^+ channel, respectively [49, 50, 78]. The maxi-channel is also more sensitive to voltage than the SK subtype. The fraction of time the maxi- K^+ channel spends in the open state, which depends on both the frequency of opening and the average duration of the open state of the channel, increases markedly with depolarization. In some tissues the maxi- K^+ channel and the SK subtype can be distinguished by sensitivities to the blockers charybdotoxin and apamin (bee venom peptide). This is also true for the anterior pituitary cell line, GH₃ cells [50], but may not be the case for neurones [49]. These blocking agents have been useful tools in the study of K^+ channels and in some cases molecular biological studies have now pinpointed the binding sites. However, these agents often affect more than one K^+ channel type [55, 66]. In addition to K^+ channel blockers a range of K^+ channel openers are known [18, 21]. The actions of these blockers on neuronal K^+ channels are not yet clear. One probable target for these agents is the neuronal ATP-sensitive K^+ channel [8].

The actions of some of the blockers have been

analysed in detail and it appears that tetraethylammonium ion and charybdotoxin are channel plugs. Interestingly there are receptors for tetraethylammonium ion on both the outer and inner aspects of the delayed rectifier K^+ channel. In squid axon, only internal tetraethylammonium is effective. In myelinated nerve, tetraethylammonium ion acts both internally and externally. A systematic analysis of K^+ channel block by tetraethylammonium ion in the squid axon was carried out by Armstrong and colleagues [3, 4, 7]. Their conclusions, which are still valid, are that the receptor or binding site for tetraethylammonium ion is in the channel beyond the gate, and that access to this site from the axoplasmic side is possible only when the channel gate is opened. The block is achieved more readily by quaternary derivatives of tetraethylammonium ion (QA), such as nonyltriethylammonium ions which have a longer hydrophobic tail. The block persists as long as the tetraethylammonium ion or QA ions are trapped beyond the gate of the closed channel, and the channel must open to allow the blocking ion to exit. These ideas are analogous to the proposals for block of the Na^+ channel by local anaesthetics and indeed preceded, and no doubt prompted, such thinking. Part of the evidence for the model of the interaction of tetraethylammonium ion with the delayed rectifier is that internal tetraethylammonium ion blocks outward K^+ current more readily than inward K^+ current. Also, external K^+ promotes the dissociation of the blocking drug from its binding site, that is inward K^+ currents reduce the block by tetraethylammonium ion. If the blocking position for tetraethylammonium ion lies within the channel beyond the channel gate, this means that the architecture of the K^+ channel would resemble that proposed for the Na^+ channel, with a wide aqueous chamber between the channel gate and selectivity filter. Block of the maxi- K^+ channel by tetraethylammonium ion has been studied also in some detail in sympathetic neurones under patch-clamp [78]. The drug is less effective internally than externally and the block-unblock process is faster (i.e. the lifetime of the tetraethylammonium ion receptor complex is shorter) with internal tetraethylammonium ion.

Role of K^+ channels

K^+ channels are a very diverse family of membrane proteins and it is clear that many of the K^+ channel subtypes can co-exist in individual neurones in the central nervous system. Fewer subtypes may be present in peripheral nerve axons. Indeed it would be interesting to know what is the distribution of the various K^+ channels in nerve cells? Are subtypes present on the somatic rather than the axonal membrane? Are different K^+ channels present on dendrites or nerve terminals? These issues remain unresolved. What is clear is that these channels have several important roles to fulfil. Some of these may be considered briefly.

First, a high K^+ conductance is responsible for the resting membrane potential. Which K^+ channels are likely to be involved? Obvious candidates are the inward rectifier K^+ channels and the Ca^{2+} -activated

K^+ systems. The inward rectifier channels (see [28]) would pass outward K^+ current at potentials close to the K^+ equilibrium potential (E_K) when the outward electrochemical gradient is small. This channel therefore may contribute to the resting potential. The Ca^{2+} -activated K^+ channels may be open at the resting membrane potential when $[Ca^{2+}]_i$ is close to normal values (i.e. 100 nmol litre⁻¹). It may become apparent that the SK channel is more important than the maxi- K^+ channel in contributing to resting K^+ conductance.

In the squid giant axon, the repolarization of the action potential is brought about by activation of the delayed rectifier. Block of this channel by tetraethylammonium ion therefore increases the duration of the action potential. In hippocampal neurones, repolarization of the action potential is slowed by both tetraethylammonium ion and charybdotoxin (a maxi- K^+ channel blocker). This raises the possibility that the maxi- K^+ channel mediates repolarization of these neurones [82]. The after-hyperpolarization after a single action potential is also reduced by charybdotoxin in the hippocampus. It may be that more than one K^+ channel subtype is responsible for repolarization in some central neurones.

Transient K^+ currents, such as the A current, are activated on depolarization, then as the channels inactivate rapidly (< 100 ms), the current decays. Such A type channels are important determinants of neuronal excitability and the action potential firing pattern also. If the A channel is blocked by 4-aminopyridine for example, the threshold current may be reduced (excitability increase) and repetitive action potential discharge may be produced. For the A channel to be effective however it is important to note that the resting membrane potential of the neurone must be sufficiently negative to remove A channel inactivation.

This certainly occurs during the after-hyperpolarization after an action potential and for this reason it was originally proposed that the A current, primed by hyperpolarization but switched on by depolarization, acted to oppose depolarizing currents and therefore controlled the rate at which the membrane potential approached the threshold for action potential generation. In this sense the A channel is setting the frequency of action potential discharge.

Other K^+ channel subtypes modulate firing patterns. These include the D channel [83], the M channel [12] and the SK channel [49]. One interesting and important property of central neurones is their ability to accommodate to a depolarizing input. This feature of neuronal behaviour has been studied in detail in motoneurones and in hippocampal neurones where the accommodation of action potential discharge is controlled by a Ca^{2+} -activated K^+ conductance (probably supported by the SK channel), and in the case of hippocampal neurones, the M conductance [58, 93]. These K^+ channel subtypes exert a braking influence on the activity of the neurone such that a repolarizing "sag" is often observed during the response to depolarization and a large multi-component after-hyperpolarization follows the response (see fig. 7). Block of these channels

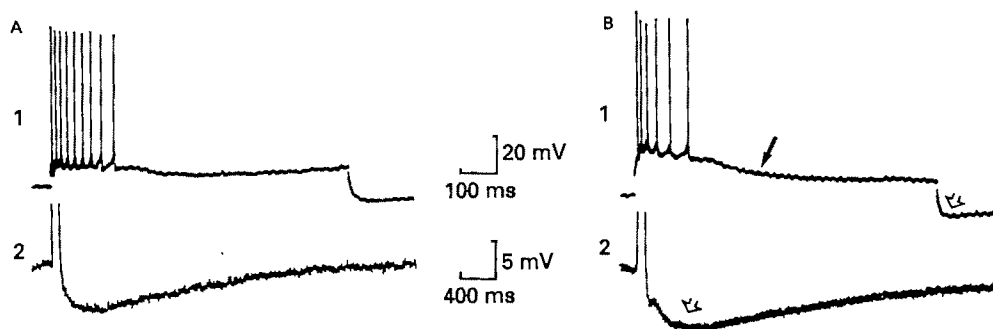


FIG. 7. The accommodation (or adaptation) of action potential discharge in central neurones. The responses of two rat hippocampal CA1 neurones to long (800 ms) depolarizing currents (A1, B1) and the after-hyperpolarizations after the responses to an 80-ms depolarizing current (A2, B2) are shown. The filled arrow in B1 indicates the typical "sag" in the voltage response. The open arrows in B1 and B2 show the after-hyperpolarizations associated with the responses. These features of the response are thought to be controlled by the M type and SK type K^+ channels.

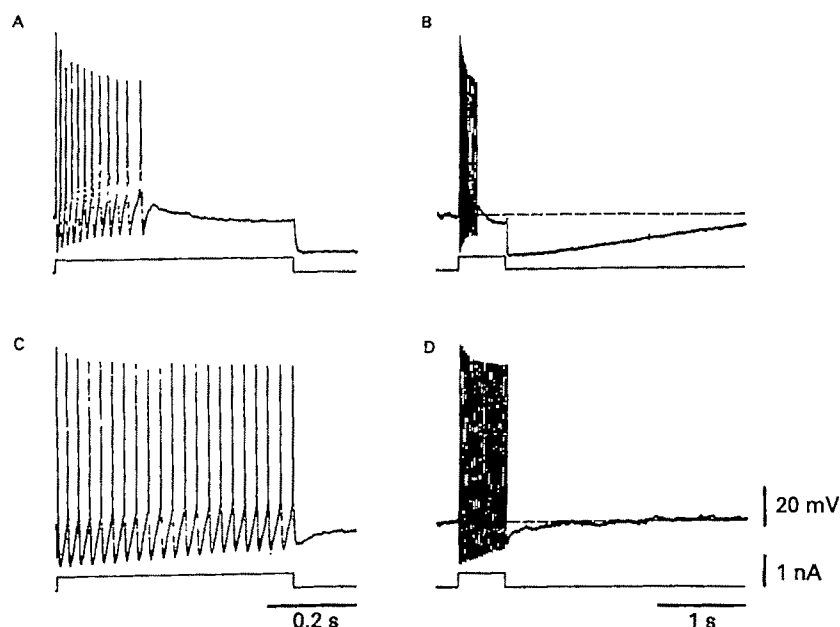


FIG. 8. Response of a rat vagal motoneurone to a long depolarizing current pulse. A and B show the control responses at two different sweep speeds, C and D show the responses at the equivalent two sweep speeds in the presence of Cd^{2+} ions $5 \text{ mmol litre}^{-1}$ to block Ca^{2+} entry. Note the abolition of accommodation of action potential discharge (C) and the reduction in the after-hyperpolarization (D) (from [93]).

results in a loss of the sag, an increase in the number of action potentials in response to a depolarizing input and a decrease in the after-hyperpolarization. This can be achieved for example by blocking the Ca^{2+} entry required to prime the Ca^{2+} -activated K^+ conductance (fig. 8), or by muscarinic agonists such as carbachol which switch off the M channel [57, 58]. These channels control the encoding properties of neurones and many transmitters and some anaesthetics can modulate encoding by modifying conductance in these channel systems.

If the appropriate K^+ channel subtypes are present in nerve terminal membranes, modulation of K^+ channel function could result in changes in transmitter release. Several K^+ channel blockers, including dendrotoxin, 4-aminopyridine and noxius-toxin enhance transmitter release [30, 77]. It may be that regulation of presynaptic K^+ channel subtypes by transmitters is a significant mechanism for controlling transmitter discharge and that this is an important physiological role for such channels.

Effects of transmitters

The properties of many K^+ channel subtypes of central neurones may be modified by transmitters. As indicated above, the M channel may be switched off by muscarinic agonists. It is also inhibited by 5-hydroxytryptamine and activated by somatostatin. The accommodation of action potential discharge and the after-hyperpolarization of hippocampal neurones is reduced by acetylcholine, noradrenaline, histamine, 5-hydroxytryptamine and dopamine (see [17, 28, 57] for references). This suggests that one class of Ca^{2+} -activated K^+ conductance (SK subtype) is under transmitter control also (i.e. switched off). The voltage-gated A channel is blocked by noradrenaline in hippocampal neurones and dorsal raphe neurones [1, 72]. Here, the A channel is coupled to beta adrenoreceptors in the hippocampus and α_1 adrenoreceptors in the raphe neurones. Neurones in the striatum and substantia nigra possess K^+ channels which are activated by D_2

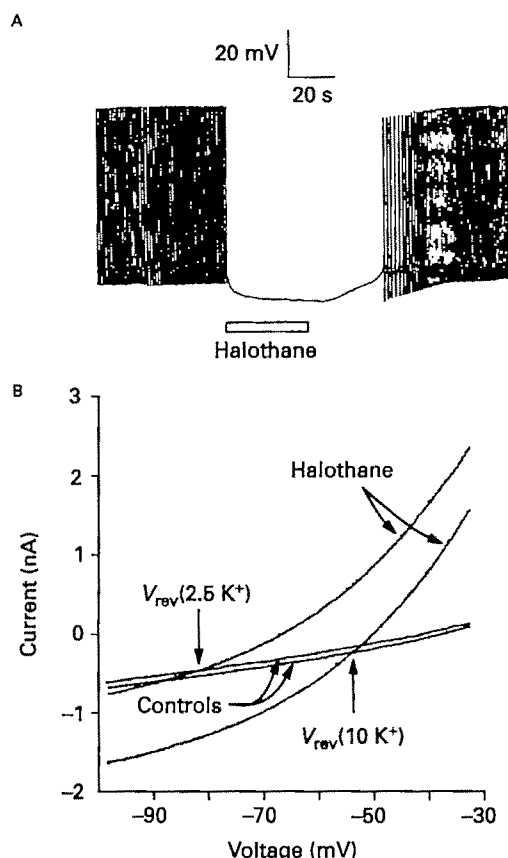


FIG. 9. A: Reversible effect of halothane (1 MAC) on the rhythmic action potential activity of a neurone in *Lymnea stagnalis*. Note the large hyperpolarization and the cessation of firing. B: Current-voltage relationship for the K^+ current activated by the anaesthetic. Data are shown at 2.5 and 10 mmol litre⁻¹ of $[K^+]_o$ in the absence and presence of halothane (from [22]).

receptor agonists [23, 69]. It is clear that many transmitter substances express their action via K^+ channel subtypes. This effect is so widespread that where the transmitters activate the conductance and induce hyperpolarization (e.g. dopamine) [69], a separate category of K^+ channel subtype, the agonist activated K^+ channel, may be proposed [28].

Effects of anaesthetics

Anaesthetics affect several different K^+ channel subtypes [80, 89, 90]. As a consequence, they increase the duration of the action potential, depolarize or, more usually, hyperpolarize and modify discharge patterns—for example reduce the accommodation of action potential discharge. Indeed it appears that in invertebrate neurones there is a class of K^+ channel which contributes to the resting conductance and potential which is particularly sensitive to volatile anaesthetics. There is an interesting difference between the effects observed in the two systems studied however. For the squid giant axon, general anaesthetics block a K^+ channel and depolarize [31]: for neurones of *Lymnea stagnalis*, anaesthetics activate a K^+ channel and hyperpolarize [22]. This latter effect, which is more common across the species (see [80, 90] for references), is shown in figure 9. Volatile anaesthetics and methohexitone often hyperpolarize hippocampal neurones, presumably by activating K^+ conductance [79, 80]. It is

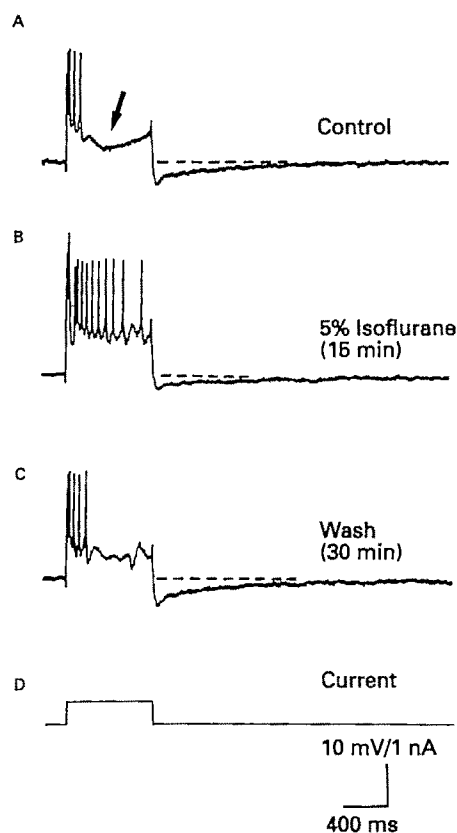


FIG. 10. Chart records of the response of a rat hippocampal CA1 neurone to an 800-ms depolarizing current pulse. A: Control response, the arrow indicates the voltage "sag" and the area beneath the dotted line is the after-hyperpolarization (AHP). B: Isoflurane (vaporizer dial setting 5%) reduces both the accommodation of action potential discharge and the AHP. C: Antagonism of the action of the anaesthetic. The AHP returns to normal, accommodation is restored partially. D: Depolarizing current pulse (from [80]).

tempting to speculate that the K^+ channel subtype is the agonist-activated K^+ channel (see above), switching on of which often produces hyperpolarization. It appears that in hippocampal neurones, at least some anaesthetics have a dual action since they can also reduce accommodation of action potential discharge and the following after-hyperpolarization. This effect is analogous to that of many transmitters and is shown in figure 10. The implication is that the activity of the M and SK type K^+ channels is suppressed by these anaesthetics. There are voltage-clamp data to support this conclusion in the case of the M channel [90]. These effects of anaesthetics on K^+ channel subtypes may underlie the changes in neuronal excitability and firing patterns often observed with such agents.

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EFFECTS OF I.V. ANAESTHETICS ON HUMAN BRAIN SODIUM CHANNELS

C. FRENKEL, D. S. DUCH AND B. W. URBAN

GENERAL CONCEPTS

The well established correlation between the potency and lipid solubility of anaesthetic compounds (Meyer-Overton correlation) has pointed to excitable membranes and membrane ion channels as possible molecular sites for anaesthetic actions, and it is now accepted widely that anaesthetics act predominantly through interactions with nerve cell membranes [46].

Many suggestions concerning detailed unitary mechanisms of anaesthetic action have been made, although a satisfactory consensus about the underlying mechanism and a single site of action has not been reached [49]. On the contrary, several specific receptors and ion channels have been identified as potential sites of action for a variety of anaesthetic agents. Therefore, it appears unlikely that a single type of channel is involved in anaesthesia. It is also conceivable that anaesthetic substances may demonstrate a multi-mechanistic mode of action at the molecular level [56]. These various investigations and the results need not be considered mutually exclusive. Abandoning the claim of unitary, many hypotheses of anaesthetic action may be seen as complementing, rather than contradicting, each other.

In this review we discuss a new electrophysiological experimental approach to investigate the molecular basis of i.v. anaesthetics on single membrane proteins from human brain cortex.

MODEL EXPERIMENTAL APPROACH: THE PLANAR LIPID BILAYER SYSTEM

In order to investigate anaesthetic interactions with individual membrane proteins, two basic electrophysiological methods are available. The patch-

clamp method and the planar lipid bilayer technique, where single membrane proteins are isolated from biological membranes and subsequently fused with an artificial lipid membrane.

In our search for the molecular basis of anaesthesia, we use a novel lipid bilayer technique, which offers the opportunity to monitor changes in the local lipid environment and to determine its effect on membrane protein function at the same time. By removing a single membrane protein from a biological membrane and inserting it into an artificial lipid bilayer of well defined composition, it may become possible to separate direct anaesthetic actions on membrane proteins from those mediated via the membrane environment.

The composition of lipid bilayers may be controlled, allowing calibrated comparisons between different membrane proteins from various tissues and species. Single molecules, after fusion with the lipid bilayer, may be observed electrophysiologically over long periods of time (many hours) with a millisecond resolution [39], permitting the measurement of control behaviour and several anaesthetic doses on the same channel. Planar lipid bilayers are sensitive detectors, requiring very little protein material. This material can be stored for years without losing its activity, providing standards for future comparison. Furthermore, many variables may potentially be controlled when using lipid bilayers: channel density, lipid composition, electrolyte composition, temperature, pH and membrane potential.

Bilayer techniques become even more powerful when isolated and purified membrane proteins are used, allowing detailed (biochemical) structure and (electrophysiological) function studies [45].

Different purified and unpurified membrane proteins (table I), such as voltage activated sodium and calcium channels and the nicotinic acetylcholine receptor, from a variety of species and tissues, have been incorporated and characterized in this experimental system (table I). This has offered, for the first time, the opportunity to study pharmacological

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KEY WORDS

Anaesthetics, intravenous: ketamine, midazolam, pentobarbitone, propofol. Human brain. Lipid bilayer. Sodium channel. Voltage-clamp.

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TABLE I. *Relevant ion channels examined in lipid bilayer membrane systems*

Membrane protein	Species	Tissue source	Purified or not	Reference
Sodium channel (voltage-activated)	Rat	Brain synaptosomes	Unpurified and purified	[37] [32] [36]
	Rat	Skeletal muscle sarcoplasm	Unpurified	[28]
	Electrophorus electricus	Electric organ	Unpurified and purified	[44] [18] [48]
	Lobster	Peripheral walking leg nerve	Unpurified	[11]
	Human	Human brain cortex synaptosomes	Unpurified	[19]
	Dog	Brain synaptosomes	Unpurified	[30]
	Rabbit	Skeletal muscle t tubule	Purified	[27]
	Bovine	Cardiac sarcolemma	Unpurified	[47]
Calcium channel (voltage-activated)		Skeletal muscle t tubule	Purified	[22]
	Rabbit	Skeletal muscle t tubule	Purified	[38]
	Rat	Brain synaptosomes	Unpurified	[40]
Nicotinic acetylcholine receptor	<i>Torpedo californica</i>	Electromotor synapse	Purified	[39]
	<i>Locust migratoria</i>	Neuronal membrane	Purified	[31]

interactions at the molecular and single channel level in a well defined and controlled membrane system.

conducted on other purified sodium channel preparations, for example the eel electroplax [60, 61].

MODEL MEMBRANE PROTEIN: THE HUMAN BRAIN VOLTAGE ACTIVATED SODIUM CHANNEL

For the investigation of anaesthetic interactions at the molecular level, the voltage activated sodium channel has been chosen as the model protein for a number of reasons, discussed elsewhere in this issue [59].

Sodium channels are ubiquitous membrane proteins essential for the generation of fast propagated action potentials and thus a key factor in neuronal signal integration and cell communication. Sodium channels have been characterized extensively in terms of electrophysiology, biochemistry, pharmacology and genetics [12], and provide an excellent model for studying anaesthetic interactions which are thought to be common to other ion channels.

Sodium channels differ not only between animal species but also between tissues in the same animal, and even between the central and peripheral nervous systems [29]. Anaesthetic sensitivities may depend on the species, location and membrane environment of sodium channels. In order to correlate clinical anaesthesia with anaesthetic actions at the molecular level, voltage activated sodium channels from human brain cortex have been studied successfully in the lipid bilayer system [19], allowing extensive electrophysiological studies of drug-protein interactions. The human brain sodium channel has been solubilized recently [50] and purified [51], revealing a protein macromolecule with a large alpha subunit (260000–300000 Dalton) and a small beta subunit (30000–36000 Dalton) [51]. This purified protein has not been functionally characterized or reconstituted into the planar lipid bilayer system. Nevertheless, as soon as this has been achieved, detailed structure-function studies in the presence of anaesthetics will be possible, such as those presently

EXPERIMENTAL DETAILS

Materials and methods

All bilayer experimental methods and materials are described in detail elsewhere [19, 24, 44]. A brief summary is given here.

After approval of the Institutional Committee on Human Rights in Research, Cornell University Medical College, New York, U.S.A., human brain cortical tissue samples were obtained from patients undergoing craniotomies for the removal of tumours (brain tumours of various histologies: glioblastoma multiforma, ependymblastoma, spongioblastoma) and healthy (accident victim, hydrocephalus operation) brain tissue. The brain tissue was normally considered surgical waste and no tissue was removed specifically or only for these experiments. Within minutes after removal, the material was stored at -80°C . After separate pooling of the tumours and healthy brain tissue, synaptosomal fractions were prepared according to the method described by Cohen and colleagues [13].

This cell fractionation technique is based on successive steps of homogenization and differential centrifugation (fig. 1). In this manner, biological ion channel-containing membranes may be broken up in such a way that the membrane fragments reseal to form small membrane vesicles. To give a perspective of the morphology of our preparation, an electron micrograph was taken (fig. 2). The synaptosomal preparation was stored at -80°C until used. This preparation has been found to be stable at this temperature for more than 4 years.

Experiments were conducted in special symmetrical Teflon bilayer chambers with 5 ml compartments as designed by Andersen [2]. The two symmetrical chambers were separated by a Teflon partition. The partition had a hole of approximately 300 μm diameter in its centre. A planar bilayer membrane,

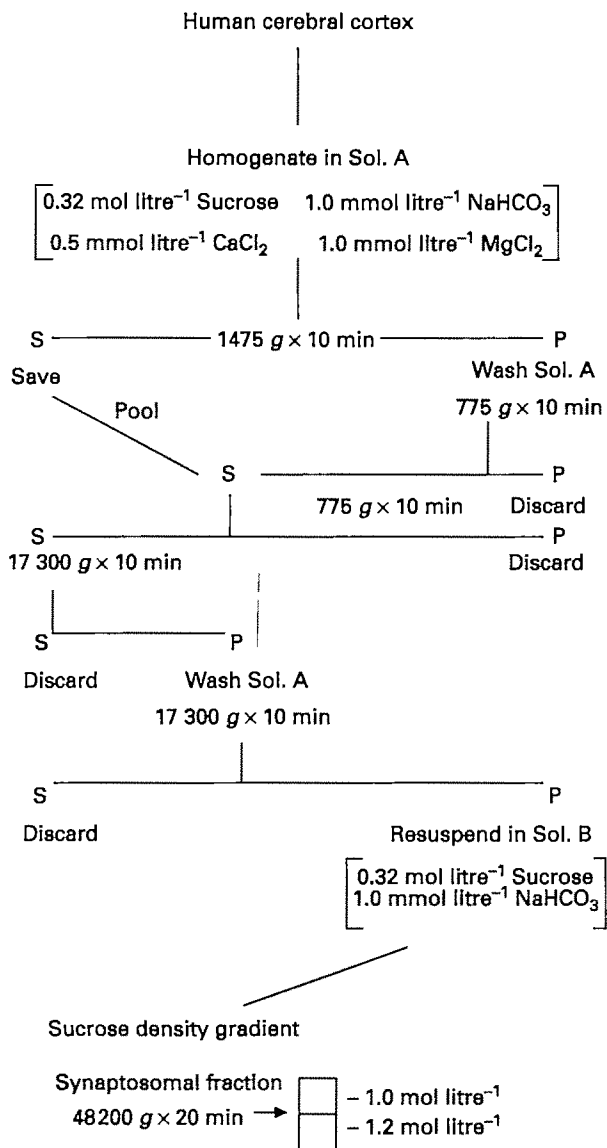


FIG. 1. Diagram of the preparation technique for human brain synaptosomal fractions.

formed across the hole of a thin Teflon partition in a bilayer chamber (fig. 3), consists of a bimolecular leaflet of lipid molecules separating two aqueous compartments. Bilayer membranes were formed from synthetic neutral phospholipid solutions, containing (4:1) phosphatidylethanolamine and phosphatidylcholine in decane (5 % w/v, 99 % pure). The chamber compartments contained symmetrical 500 mmol litre⁻¹ solutions of NaCl, buffered at pH 7.4. These two half-chambers may be conveniently viewed as corresponding to the extra- and intracellular compartments, separated by the artificial lipid bilayer representing a simplified model of a biological nerve membrane. The incorporation of sodium channels into the bilayer was achieved by adding synaptosomal vesicles close to the preformed bilayer membrane at large membrane potentials. Probably through a process of membrane fusion [41], sodium channels were transferred from vesicles into the planar lipid bilayer (fig. 4). Experiments were conducted at room temperature (22–25 °C). No corrections were made for temperature differences between individual experiments.

Sodium channels were incorporated in the presence of batrachotoxin 1 µmol litre⁻¹ added to the vesicle free bilayer chamber; this alkaloid toxin is commonly used in bilayer experiments [8] to study the steady state properties of sodium channels by removing the inactivation and increasing the open times.

In order to detect the presence of ion channels, membrane potentials were applied across the bilayer and the resulting ion flow monitored (fig. 3). Channel currents were recorded while holding the membrane potential constant, using a standard current-to-voltage amplifier. Currents were filtered at 50 Hz. Channel orientation was determined by channel gating characteristics and/or tetrodotoxin block, allowing the electrophysiological sign convention in the presentation of the results. All membrane



FIG. 2. Electron micrograph of the human brain synaptosomal fraction (initially frozen at –80 °C) preparation from healthy human brain tissue with a synaptosome in the centre of the photograph, which contains mitochondria and numerous clear synaptic vesicles (magnification 1:40 000).

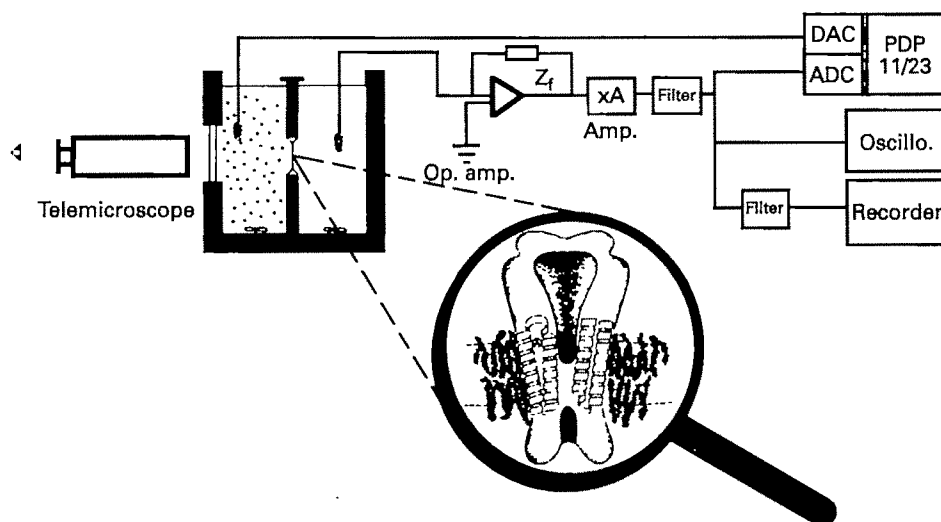


FIG. 3. Schematic diagram of the experimental set-up. A thin Teflon partition separates two electrolyte chambers, each containing a silver chloride electrode. The Teflon partition is perforated by a hole of 0.3 mm diameter (diameter exaggerated in drawing) across which the lipid bilayer is formed. The sodium channel preparation is added to one of the bilayer chambers. The membrane potential is controlled through the DAC output of the interface which is connected to a Digital Equipment LSI 11/23 computer. The other electrode records membrane currents with the aid of a current-to-voltage converter, the output (Op.) of which is further amplified (Amp.) and filtered. The resulting signal may be viewed on an oscilloscope (Oscillo.) and a chart recorder, but it is also sent to the ADC port of the interface, digitized and stored for subsequent analysis. In the magnifying glass, a sodium channel already incorporated in the planar lipid bilayer is shown.

potentials are quoted as intracellular minus extracellular potentials. Under these steady state conditions, the same channels could be observed for many hours.

For the measurement of fractional open times, current traces were recorded by computer, time-averaged and, after subtraction of the membrane capacitive transients, converted into conductances. The fractional open time, f_o , was obtained by subtracting from the averaged conductance the background conductance through the lipid bilayer

and dividing by the number of channels and the single channel conductance [19]. The fractional open time is dimensionless, representing the fraction of time that a channel is open during the observation period.

To measure voltage-dependent steady state activation characteristics, membrane potentials were changed using a hyperpolarizing potential sequence of -10 -mV steps from a holding potential of $+50$ mV until the channel was fully closed. For some experiments, the membrane was then depolarized sequentially in $+10$ -mV steps back to $+50$ mV. Each potential was held for 4 s. The first 2.5 s of each trace were used to calculate fractional open times. The series of fractional open times resulting from such a potential sequence were plotted as a function of membrane potential and represented the steady state activation response. Subsequently, these fractional open times were fitted to a two-level Boltzmann distribution by a least squares fitting procedure [44]. The fractional open time, plotted as a continuous function of membrane potential, is referred to as the steady state activation curve. This curve is characterized by the midpoint potential (V_a) at which the channel spends 50 % of the time in the closed and 50 % in the open state and by the effective gating charge (z_a). The product $z_a \cdot V_a$ is related to the free energy difference between the voltage-gated open and closed state of the channel. In practical terms, z_a is also a useful quantitative measure of the steepness of the steady state activation curve, that is the sensitivity with which the channel activation mechanism responds to changes in membrane potential.

After channel incorporation and control measurements, the i.v. general anaesthetics were added to the aqueous phase. After addition of drug, the aqueous

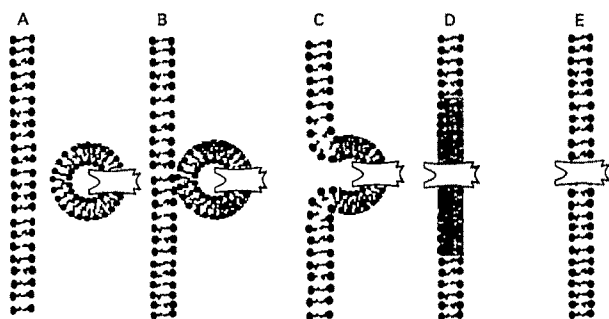


FIG. 4. Schematic diagram of the fusion process of a sodium channel containing vesicle with the planar lipid bilayer. A: Essential features of the lipid bilayer membrane with phospholipids are indicated. Close to the membrane, a synaptosomal vesicle containing a sodium channel protein. The thickness of the bilayer membrane is approximately 4 nm. B: Next step of the fusion process: adhesion of the vesicle to the bilayer. C: Start of the direct fusion: vesicle and membrane are opening up and the vesicle membrane, including the sodium channel, is integrated into the bilayer membrane. D: The fusion process is completed and the sodium channel protein is inserted in the artificial lipid bilayer. The hatched bilayer area indicates the vesicle membrane portion. E: In the final step, the incorporated functioning sodium channel is located in a membrane where the vesicle membrane lipids are no longer discernable as they have mixed with the much larger pool of bilayer lipids.

compartments were stirred for 10 s to ensure homogeneous drug dilution. In order to avoid depletion of the anaesthetics from the aqueous phase, large aqueous compartments (4 ml) were used, while lipid phases were deliberately kept small (typically less than 0.15 μl).

Results

Under control conditions, human brain sodium channels showed stable and reproducible characteristics, as expected for batrachotoxin modified sodium channels in bilayers [19, 24]. Single channels were identified as sodium channels by their single channel characteristics (conductance, fractional open time, steady state activation behaviour) and in some cases by their sodium selectivity and block by tetrodotoxin, a sodium channel specific inhibitor [19, 44].

Sodium channels were monitored for 30–40 min before any anaesthetic was added to the bilayer chambers [29], during which the channels remained mostly open at depolarized potentials (fig. 5) with a fractional open time of 0.94 (SD 0.04) ($n = 57$ membranes). After membrane hyperpolarization, human brain sodium channels in bilayers closed with an average midpoint potential of -84 (10) mV ($n = 71$). The effective gating charge (measure of the steepness of the activation curve) was 2.7 (1.7).

After the addition of anaesthetic agents to the extra- or intracellular electrolyte in the presence of the same control channel, an increasing number and duration of channel closing events could be observed, so that the channel spent more time in the closed state (fig. 5). The electrophysiological measurements were repeated and continued until the membrane broke spontaneously or additional incorporations occurred. Channels in membranes exposed to pentobarbitone (163 mins, $n = 14$ experiments) and ketamine (149 mins, $n = 14$) could be observed much longer, on average, than in the presence of propofol (39 mins, $n = 9$) or midazolam (36 mins, $n = 21$). Pentobarbitone (fig. 5A) and ketamine produced a

"flickery" appearance of the current traces with a transition frequency of greater than 50 Hz. Longer closures and brief transitory closures were observed with propofol and midazolam (fig. 5B). The noise level and background conductance detected during prolonged channel closures was not different from that during control measurements, which indicated that all anaesthetics by themselves had no effect on the conductance and stability of lipid bilayer membranes.

Increasing doses of the anaesthetics caused an increased number and duration of channel closing events and time-averaged currents were suppressed increasingly. As this suppression of current was linear between membrane potentials of $+45$ mV and -45 mV, time-averaged conductances were calculated from these data and averaged over this membrane potential range. Anaesthetic drugs introduced to either side of the channel gave similar results, so the data were pooled.

From the resulting dose-response curves (fig. 5) weighted least-square computer fits produced values for the half-maximal suppression concentration (ED_{50}) and the maximal conductance block (max block %). An ED_{50} value of $0.69 \text{ mmol litre}^{-1}$ and a max block of 100 % was calculated for pentobarbitone. For propofol, the ED_{50} was $0.02 \text{ mmol litre}^{-1}$ and the max block was 28 % (fig. 5). The dose-response curves for racemic ketamine and midazolam yielded an ED_{50} of $1.1 \text{ mmol litre}^{-1}$ and a max block of 71 % for racemic ketamine, and $0.52 \text{ mmol litre}^{-1}$ and 100 % for midazolam, respectively.

Although conductance suppression was similar for all four drugs, only pentobarbitone and propofol produced their effects at concentrations close to those relevant to human anaesthesia and long-term sedation (table II), indicating different sodium channel specificities of the investigated drugs.

The hallmark of a voltage operated ion channel is the dependence of its opening and closing behaviour on membrane potential. Under physiological con-

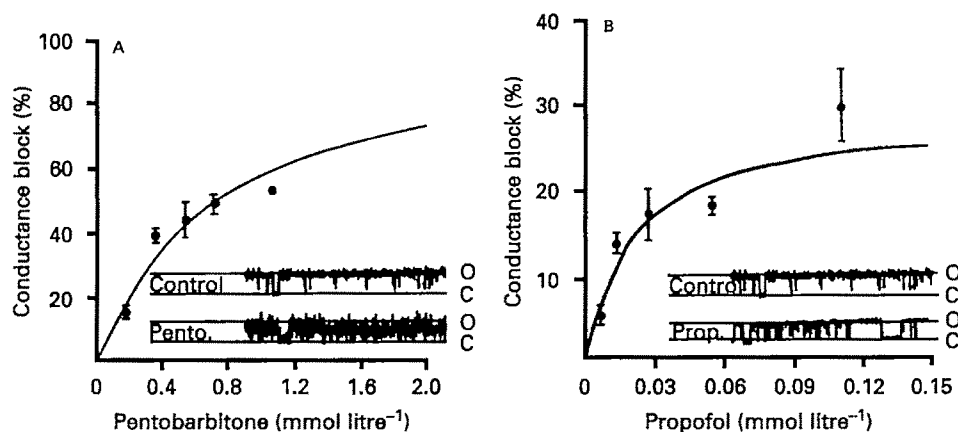


FIG. 5. Conductance block expressed as the fraction of the average conductance reduction and control conductance, plotted as a function of anaesthetic concentrations. Data (mean, SEM) have been pooled for membrane potential between -45 mV and $+45$ mV. Weighted computer fitted concentration-response curves yielded an ED_{50} of $0.69 \text{ mmol litre}^{-1}$ and a maximal conductance block of 100 % for pentobarbitone (A) and $0.02 \text{ mmol litre}^{-1}$ and 28 %, respectively, for propofol (B). In the inset, the original current traces from a single batrachotoxin activated single human brain sodium channel at -45 mV membrane potential at control conditions and after addition of $0.67 \text{ mmol litre}^{-1}$ of pentobarbitone (A) or $0.056 \text{ mmol litre}^{-1}$ of propofol (B) are shown. O = the fully open, C = the fully closed channel level. Records were filtered at 50 Hz (8-pole Bessel).

TABLE II. Clinically relevant peak serum and cerebrospinal fluid (CSF) concentrations of various i.v. anaesthetics in humans. † = Peak CSF concentration ($\mu\text{g ml}^{-1}$); ‡ = peak CSF concentrations ($\mu\text{mol litre}^{-1}$)

Anaesthetic	Application mode	Dose	Peak serum conc.			
			$\mu\text{g ml}^{-1}$	$\mu\text{mol litre}^{-1}$		
Hypnotics: barbiturates						
Pentobarbitone	I.v., premedication	100 mg bolus	3.5	[20]	15	
	I.v., long term sedation	30 mg kg ⁻¹ bolus, 3 mg kg ⁻¹ h ⁻¹ maint.	50	[3]	200	
Thiopentone	I.v., induction	6 mg kg ⁻¹ bolus	100	[9]	380	
	I.v., long term sedation	150 mg bolus, 150 mg h ⁻¹ maint.	20	[10]	80	
Methohexitone	I.v., sedation	3 mg kg ⁻¹ over 60 min	4	[7]	15	
	I.v., long term sedation	24 mg kg ⁻¹ over 60 min	25	[55]	90	
Hypnotics: non-barbiturates						
Propofol	I.v., total i.v. anaesthesia	2 mg kg ⁻¹ bolus, 0–10 mg kg ⁻¹ h ⁻¹ variable maint.	6.0	[54]	35	
	I.v., long term sedation	3 mg kg ⁻¹ h ⁻¹	3.5	[4]	20	
Etomidate	I.v., total i.v. anaesthesia	150 mg/30 min	2.5	[52]	10	
Benzodiazepines						
Diazepam	I.v. sedation	20 mg bolus	0.6	[35]	2	(NA)† (NA)‡
	I.v., long term sedation	30 mg day ⁻¹ , 14 days	1.5	[58]	5	(0.13 [34])† (0.46)‡
Flunitrazepam	I.v., sedation	0.02 mg kg ⁻¹ bolus	0.027	[1]	0.1	(0.002 [1])† (0.001)‡
Midazolam	I.v., sedation	0.07 mg kg ⁻¹ bolus	0.1	[6]	0.2	(0.023 [6])† (0.05)‡
	I.v., induction	0.3 mg kg ⁻¹ 3 × bolus every 30 min	1.3	[15]	3	(NA)† (NA)‡
	I.v., long-term sedation	5 mg bolus, 4–14 mg h ⁻¹ maint.	4.1	[17]	10	(NA)† (NA)‡
Fentanyl	I.v., induction	0.01 mg kg ⁻¹ bolus	0.09	[5]	0.2	
Sufentanil	I.v., induction	0.002 mg kg ⁻¹ bolus	0.06	[44]	0.2	
Alfentanil	I.v., induction	5 mg bolus	0.8	[53]	2	
Various						
Droperidol	I.v., sedation	5 mg bolus	0.8	[14]	0.2	
		0.15 mg kg ⁻¹ bolus	0.5	[21]	1.3	
Ketamine	I.v., induction	2 mg kg ⁻¹ bolus	5	[62]	20	

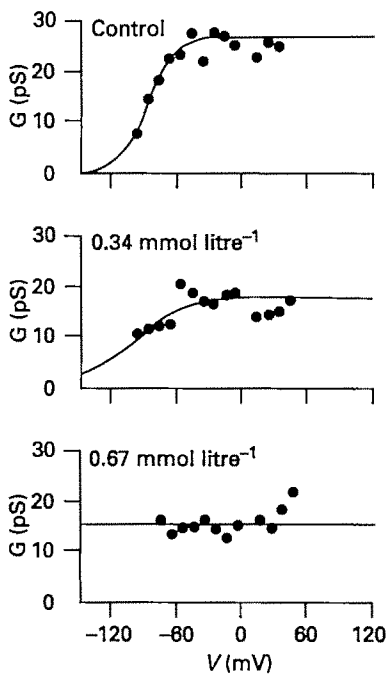


FIG. 6. Averaged steady state activation responses recorded from the same single sodium channel at increasing (+)-pentobarbitone concentrations. Control (average of two activation curves), 0.34 (average of three curves) and 0.67 mmol litre⁻¹ (single activation curve.)

ditions in a nerve cell, sodium channels are closed at rest and open when the membrane is depolarized, for example when an action potential is initiated. While the results reported so far were independent of membrane potential, all anaesthetics disrupted steady state activation also (i.e. the voltage-dependent

opening and closing of sodium channels). The batrachotoxin modified human brain sodium channels also exhibited this behaviour [19]. Under control conditions, human brain sodium channels in bilayers could be closed with sufficiently large, negative membrane potentials (average midpoint potential -84 (10) mV). These steady state activation curves were reproducible during all control determinations [19, 23, 24].

Anaesthetic treatment of the observed sodium channels introduced a noticeable difference compared to control channels; the variability of the response to changes in membrane potential was altered. This variability in the gating behaviour was demonstrated by different successive steady state activation responses of the same channel [57].

In most, but not all, cases sodium channels treated with pentobarbitone [24], ketamine [26] and propofol [25] could be closed by sufficiently large negative membrane potentials. The slopes and shapes of the resulting curves could vary substantially and abrupt changes in steady state activation responses for the same channel over time were prominent.

In the traditional macroscopic electrophysiological measurement, many channels would reside within the same membrane and only their average behaviour could be observed. In order to mimic "macroscopic" behaviour of these single channels, the fractional open times obtained during multiple determinations of the steady state activation responses of the same channel were added at each potential and divided by the number of determinations. The resulting average steady state activation response was relatively smooth, and the variability manifested itself only in a reduced slope of the subsequently fitted steady

state activation curve [23–26]. Thus it became obvious that measurements of macroscopic currents would have given no hint as to the complexity of the anaesthetic actions at the molecular level. Furthermore, an apparent shift of the activation midpoint potential to more hyperpolarized potentials was indicated [24–26].

This effect appeared to be dose-dependent also. Figure 6 shows steady state activation curves from a single sodium channel which was exposed to increasing doses of pentobarbitone.

The anaesthetic-induced effect of conductance suppression and a successively reduced steady-state activation slope became more prominent with increasing pentobarbitone concentrations. The same effect could be demonstrated for racemic ketamine [26].

DISCUSSION

The present studies demonstrate two major impacts by all investigated anaesthetic compounds on the batrachotoxin modified sodium channel: voltage-independent reduction of the fractional channel open time and interaction with voltage-dependent steady state activation. The possibility of anaesthetic actions being altered in the presence of batrachotoxin, which affects voltage-dependent sodium channel activation and inactivation, and also selectivity and single channel conductance [8], has been discussed previously [24] but needs to be investigated further. Qualitatively, the finding of more than one type of anaesthetic action on single sodium channel currents for the investigated i.v. general anaesthetics is in line with previous studies of volatile gaseous anaesthetics on the macroscopic scale with peripheral nerve [33, 56], performed without batrachotoxin. The anaesthetic-induced variability in steady state activation behaviour, however, suggests that anaesthetic actions at a molecular level may be

much more complex than indicated in macroscopic measurements [46].

Pentobarbitone and propofol exhibited their effects on human sodium channels at concentrations nearer clinically relevant free serum concentrations (see table II) than did ketamine and midazolam, which produced their effects at concentrations approximately 100-fold greater than relevant free serum concentrations (table II). Free (protein unbound) serum concentration of anaesthetics may not be a true reflection of that at *in vivo* sites of action, but it is the only estimate presently available. Information about anaesthetic concentrations in cerebrospinal fluid or brain tissue of humans during anaesthesia or long-term sedation is lacking for most substances (see table II).

The human CNS sodium channel may therefore serve as a potential target site of action for propofol and pentobarbitone. As ketamine and midazolam produce their effects on the sodium channel only at concentrations well beyond the relevant drug concentration range, other membrane proteins and receptors may be involved in their anaesthetic action, supporting our hypothesis that anaesthesia results from the superposition and integration of several anaesthetic actions on the molecular level [56]. This diversity is consistent with clinical observations, as propofol and pentobarbitone, when used as induction agents, display similar hypnotic characteristics, which are different from the dissociative or sedative types of anaesthesia produced by ketamine or midazolam, respectively. This suggests that different types of clinical anaesthesia may correlate with differential actions of anaesthetics on the molecular level.

Meyer and Overton showed a close correlation between lipid solubility and anaesthetic potency for volatile and inhalation anaesthetics. Figure 7 also shows a good correlation between the free (protein unbound) serum concentrations of five hypnotic agents (three barbiturates, propofol and etomidate) and lipid solubility. When a wider range of i.v. anaesthetics is considered, a reasonable correlation (slope = -0.69 , regression coefficient = 0.90) exists between anaesthetic half-maximal blocking concentration of human brain sodium channels, representing the experimental anaesthetic potency, and the octanol:buffer coefficient of the drug as a measure of lipid solubility (fig. 8A). This correlation becomes stronger when only the experimental anaesthetic potencies of the hypnotic agents (pentobarbitone, propofol and etomidate) are considered (slope = -0.87 , $r = 0.95$) (fig. 8B).

Although these sodium channel half-block concentrations are approximately 10-fold greater (fig. 8B) than those encountered in the clinical situation (fig. 7), a significant contribution of lipophilic interactions to the mechanism of anaesthetic action of these substances need not be ruled out. The local concentrations of anaesthetics at the site of actions may be increased by some additional polar interactions that by themselves may have no anaesthetic effect.

One can only speculate as to the site and exact mode of interaction of these different anaesthetics

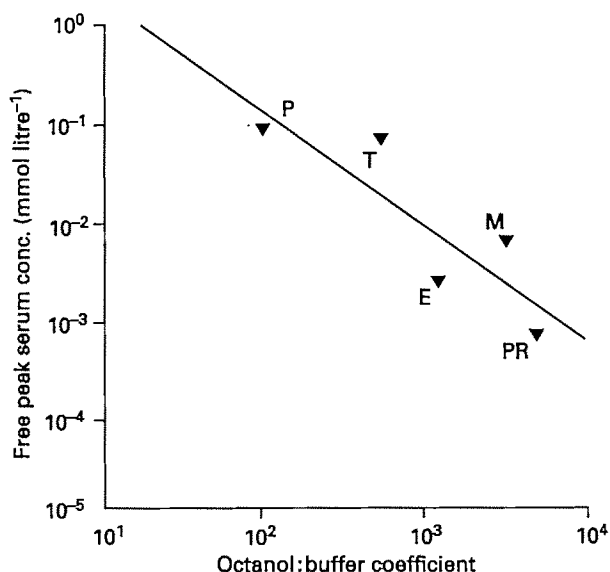


FIG. 7. Double logarithmic plot of the free (protein unbound) peak serum concentration of five hypnotic induction agents (pentobarbitone (P), thiopentone (T), methohexitone (M), etomidate (E), propofol (PR)), as a function of octanol:water partition coefficients. A good correlation with lipid solubility was found (slope = -1.18 , $r = 0.86$).

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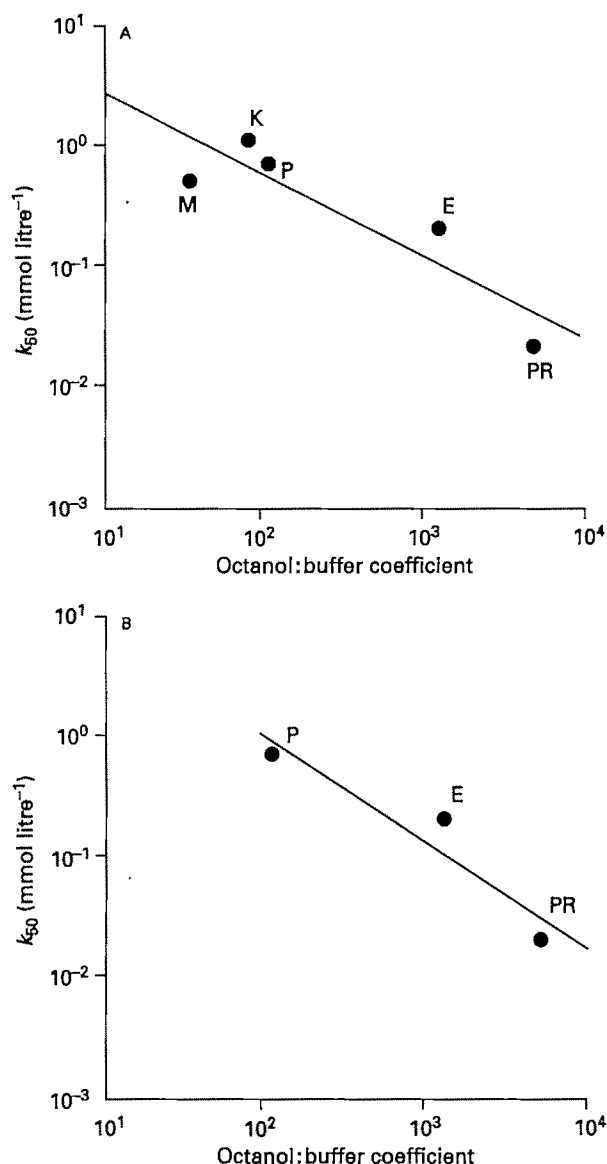


FIG. 8. A: Double logarithmic plot of anaesthetic half-maximal blocking concentration (k_{50}) of human brain sodium channels for ketamine (K), pentobarbitone (P), midazolam (M), etomidate (E), propofol (PR) as a function of lipid solubility (octanol:buffer coefficient at room temperature and pH 7.4). Slope = -0.69 , $r = 0.90$. Data for etomidate unpublished [Weckbecker, personal communication]. B: Double logarithmic plot of the half-maximal blocking concentration (k_{50}) of human brain sodium channels of the three hypnotic induction agents (pentobarbitone (P), propofol (PR), etomidate (E)) as a function of lipid solubility (octanol:buffer coefficient at room temperature and pH 7.4). Slope = -0.87 , $r = 0.95$.

with the sodium channel. The close correlation between lipid solubility and anaesthetic potency points to a lipophilic site of anaesthetic action, which need not be limited to the lipid bilayer. The amino acid sequences of sodium channel proteins are known and some features of the transmembrane structure of the channel have been proposed (fig. 9). They share many segments of probable alpha helical structure [12, 16], with many strongly hydrophobic segments; certain amphipathic regions, which are both hydrophobic and positively charged, are thought to be involved in channel steady state activation. Anaesthetics may penetrate into the areas where the helices contact each other, thus distorting protein

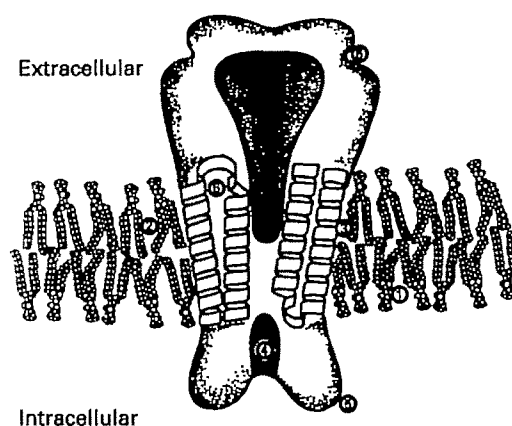


FIG. 9. Schematic diagram of a voltage-activated sodium channel imbedded in a lipid bilayer membrane. Potential sites of anaesthetic action include: (1) lipid membrane surface; (2) lipid membrane inner portion; (3) lipid membrane-protein interface; (4) protein-ion pathway; (5) protein intra-, extracellular space; (6) protein inner segments (alpha helix).

conformation and channel function. This could lead to the dramatic increase in transitions between open and closed states, and the substantially increased variability of the steady state activation responses in the presence of all the anaesthetic agents observed in our experiments. Thus anaesthetics may increase protein flexibility (i.e. the number of accessible conformational states). They may interact also with a multitude of other sites: the lipid membrane surface, the lipid membrane inner portion, the lipid-protein interface (contact region), the ion pathway within the protein and the extra- or intracellular side of the protein (sugar residues) (fig. 9).

Lipophilic interactions may be very important in the actions of i.v. hypnotic agents, such as barbiturates, etomidate and propofol. The correlation between lipid solubility and experimental anaesthetic potency on sodium channels gives an indication that the lipid bilayer system may be a valid model system to quantitate the lipophilic or hydrophobic component in the mechanism of action of any particular anaesthetic.

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DIFFERENTIAL EFFECTS OF GASEOUS AND VOLATILE ANAESTHETICS ON SODIUM AND POTASSIUM CHANNELS

B. W. URBAN

After 150 years of clinical anaesthesia, the molecular events that lead to anaesthesia have not been determined. This is not because of a lack of ideas [16–19, 56, 57]. However, most striking to the observer is the paucity of long-term and systematic studies. The present review describes results from such a study. The long-term goals were: first, to understand how anaesthetics affect membrane protein function and second, to determine if clinical and molecular actions of anaesthetics may be correlated.

Definition of anaesthesia and anaesthetic

Before the mechanisms of anaesthetics are discussed, the term “anaesthesia” should be defined in practical terms [64]: “Anaesthesia describes the condition of a patient corresponding to the one produced by diethyl-ether which permits surgery to be performed without the patient moving, reacting to pain, or remembering the surgical intervention after recovery from anaesthesia.”

Strictly speaking, only a substance that has all these qualities may be called a general anaesthetic. However, clinical practice is such that almost all general anaesthetics are given in combination with other agents at concentrations where, by themselves, they may not produce anaesthesia. Therefore, the term general anaesthetic is used for substances that, at sufficiently large concentrations, could produce anaesthesia, although for a variety of reasons these large concentrations are not used in common clinical practice. While this definition extends across the range of general anaesthetics, basic scientists have broadened the definition further: they include all substances that in animal experiments produce “anaesthesia-like” states, as determined by the absence of certain reflexes (e.g. in response to pain, in response to posture changes, etc), and in addition, anaesthetic-like compounds are included also. These substances may not have been tested in whole animal experiments, but may act similar to general

anaesthetics when used in *in vitro* preparations, at the network, cellular, subcellular or molecular level.

Integrative approach towards understanding anaesthetic action

The action of an anaesthetic at a particular molecular site will depend on how this molecular site is integrated into the function of the CNS. The anaesthetic effect on a particular membrane protein (e.g. a sodium channel) is the result of several and simultaneous actions at presumably distinct sites of the sodium channel macromolecule. The total anaesthetic response of a neurone again results from the summation of the responses of its different ion channels and ion pumps to the anaesthetic and the various inputs it receives from neurones of surrounding networks (fig. 1).

The example in figure 1 illustrates the complexity of possible anaesthetic modified responses of a neurone, although the example is oversimplified, since a typical neurone within the cortex receives thousands of synaptic inputs [38]. Most neurones, even when exposed to an anaesthetic, will not respond to an incoming signal on a single input (fig. 1A and 1E), unless some temporal (fig. 1B) or spatial (fig. 1C) integration has taken place that has not been offset by a simultaneous inhibitory input (fig. 1D). Anaesthetic modifications of the incoming signal (which need not be blocked completely) that prevent a neurone from firing may involve alterations of firing rates (fig. 1F) or temporal shifts (fig. 1G) in the incoming signals. Alternatively, a temporal shift involving inhibitory inputs may lead to removal of inhibition (fig. 1H). A network of neurones responds with integration of the anaesthetic responses from individual member neurones. Partial inhibition may lead to complete inhibition at the next higher level of integration, alternatively, it may result in removal of inhibition (fig. 1). It is not possible to predict the effects of anaesthetic actions at the molecular level without knowing the neuronal network topology. Conversely, knowledge of the network topology will not allow predictions about anaesthetic effects on the CNS, without understanding of anaesthetic actions at the molecular level.

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KEY WORDS

Action potential. General anaesthetic. K⁺ channel. Lipophilicity. Mechanism of action. Membrane excitability. Meyer–Overton correlation. Na⁺ channel.

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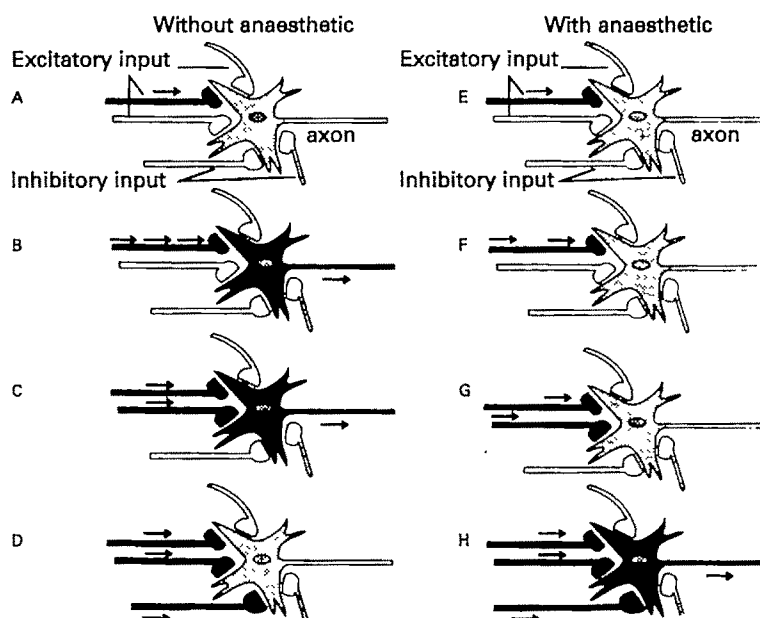


FIG. 1. Spatial and temporal integration of excitation and inhibition within a model synapse and its potential modification by anaesthetics. Resting fibres are drawn white, grey indicates a non-propagating excitation, black symbolizes a propagating excitation. The number of arrows indicates the frequency of incoming signals, their relative shift a temporal shift in incoming signals (see text for explanation).

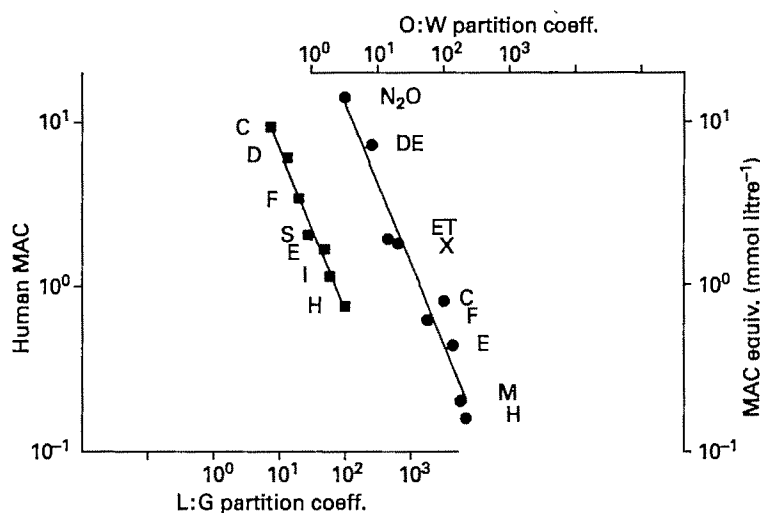


FIG. 2. Left: Meyer-Overton correlation of the logarithms of human MAC values for cyclopropane (C), desflurane (D), fluroxene (F), sevoflurane (S), enflurane (E), isoflurane (I) and halothane (H) vs the logarithms of the lecithin:gas (L:G) partition coefficient (slope -0.987). Data from Taheri and colleagues [61], who could not find a statistically significant difference between the correlations of anaesthetic potency with either lecithin:gas or octanol:gas partition coefficients. Since there are more partition coefficient data available for the octanol:water system and to facilitate later comparisons with i.v. anaesthetics, water:octanol coefficients may be used equally as well. Right: Human MAC data converted into equivalent aqueous concentrations [66] vs octanol:water (O:W) partition coefficients [56] (slope -0.991). DE = Diethyl ether; ET = ethylene; X = xenon; M = methoxyflurane.

Identifying key anaesthetic interactions

A vast range of physiological changes and responses are observed in an organism under anaesthesia and various stages of anaesthesia are distinguished clinically. Separate molecular mechanisms may account for the many different manifestations of anaesthesia, in much the same way as separate mechanisms have been found responsible for the different forms of sleep and memory [38]. The large variety of anaesthetic responses could result from a combination of several types of anaesthetic interactions with different proteins and

membranes. It is thus important to identify and understand the nature of these key anaesthetic interactions, and appropriate model systems have to be used in which anaesthetic interactions may be studied systematically.

Meyer-Overton correlation

The Meyer-Overton correlation (fig. 2) shows that anaesthetic potency and lipid partition coefficients are related linearly over many concentration decades [45]. This finding pointed to membranes as an important site of action of anaesthetics [54]. This simple relationship indicated that the underlying

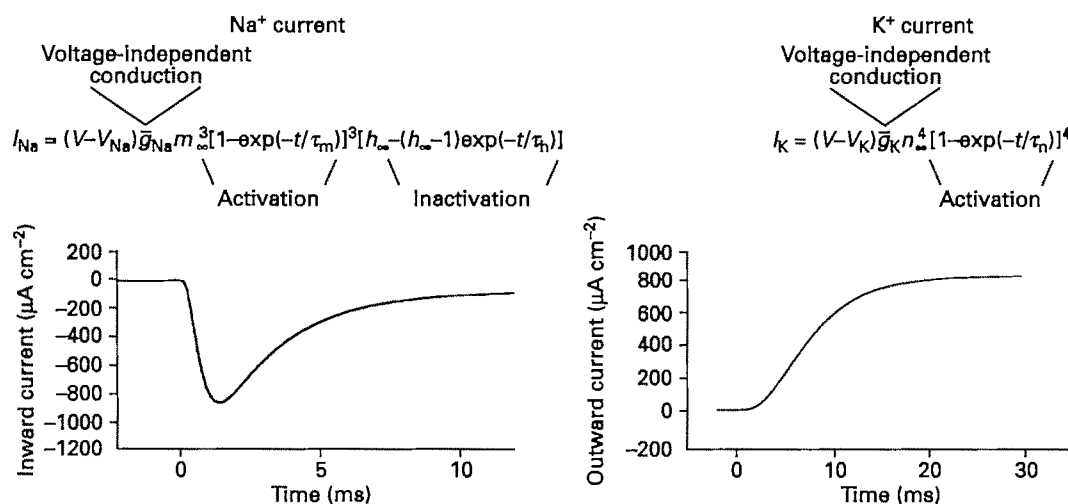


FIG. 3. According to Hodgkin-Huxley formalism [36], sodium currents are the product of three different terms which describe membrane potential-independent conductance ($\bar{g}_{Na}$), activation and inactivation. V = membrane potential, V_{Na} and V_K = sodium and potassium equilibrium potentials, respectively. The activation term contains a steady state variable (m_∞), the cube of which describes the fraction of channels activated in the steady state and an exponential expression including a time constant (τ_m). Potassium currents are a combination of a membrane potential-independent conductance ($\bar{g}_K$) and an activation term containing a steady state variable (n_∞) the fourth power of which describes the fraction of channels activated, and an exponential expression including a time constant (τ_n). All variables, except for $\bar{g}_{Na}$ and $\bar{g}_K$, are functions of membrane potential, that is they assume different values at different membrane potentials (see fig. 5).

mechanism was simple also. Yet, while sharing this common characteristic of lipophilicity, anaesthetic molecules were found to vary greatly in their chemical and physical properties [48, 59]. Thus this suggested a non-specific mode of action, comparable to the action of an antifreeze in a car radiator where depression of the freezing point results from disruption of the ice bonding structure, and depends only on the number of molecules of antifreeze and not on its particular chemical structure. It was thought that anaesthetics dissolve in membranes and thereby disrupt the structure of the membrane. However, an important question remained: how could changes in these physical variables impair the electrical excitability of a membrane?

Membrane proteins and ion channels

Lipid bilayer membranes, consisting of a bi-molecular leaflet of lipid molecules, constitute the backbone of a biological membrane. Lipid bilayers are not electrically excitable as they are perfect insulators, permitting no ion flow. However, biological membranes also contain many different membrane proteins which are essential for mediating many physiological functions. Direct or indirect interference of anaesthetics with membrane proteins is likely to be of great importance. Opinions have been divided on whether or not anaesthetics interfere with membrane protein function by binding directly to proteins [20, 54] or if the main modes of action occur indirectly through changing the physico-chemical properties of the lipid membrane into which the anaesthetics readily dissolved [15, 46]. In order to differentiate to what extent anaesthetics interfere with the interaction between lipids and membrane proteins, while possibly also interacting with membrane proteins directly, it is important to select appropriate membrane proteins for these studies and to monitor the effects of systematically

changing the structure of membranes, proteins and anaesthetics alike.

Ion channels constitute a class of membrane proteins that render membranes electrically excitable [35] but also susceptible to the actions of anaesthetics [57]. The study of ion channels is therefore of both immediate relevance, and wider interest within the context of characterizing the interactions between anaesthetics, lipids and integral membrane proteins. Because in-depth studies of ion channels are time-consuming and difficult [15, 47, 65], it is not practical to abandon one and begin a new study every time fashions and ideas change as to what ion channel is the most important for anaesthesia [49]. In order to characterize the anaesthetic interactions that different ion channels have in common, a good starting point is a thorough investigation of typical and well characterized ion channels that may serve as model channels.

Sodium channel as a model system for ion channels

Voltage-gated sodium channels are obvious and strong candidates to represent a model system. With the possible exception of acetylcholine receptors, sodium channels are unparalleled in the extent to which their structural and functional properties have been characterized [5, 35, 39]. They are ubiquitous in the central and peripheral nervous system, mediating fast propagated action potentials, which are vital to nerve impulse transmission and cell communication [38]. Sodium channels play an important role in signal integration; action potentials are initiated in the axon hillock region where the density of sodium channels is large [38].

Sodium channel function, using voltage-clamp, patch-clamp, lipid bilayer and ion flux techniques, has been described by detailed mathematical models and formalisms (fig. 3) [3, 36, 44, 52]. Their structural properties have been, and are being,

elucidated in numerous studies using biochemistry, spectroscopy and molecular biology techniques [1, 6, 8, 42, 51]. Ion channels may be grouped into families of evolutionary and structurally related gene products; for example, 55% sequence homology exists between sodium and calcium channels [8, 62]. Because sodium channels have served as a model for the description of almost all other voltage-dependent ion channels (e.g. fig. 3) and with suitable modifications even for chemically activated channels [35], they provide a good starting point for the study of anaesthetic interactions with ion channels. The actions of hundreds of agents, including anaesthetics, have been evaluated in pharmacological studies [50, 55, 59]. The suppression of sodium channels by gaseous and volatile anaesthetics follows the Meyer-Overton correlation [63].

Mechanistic basis for action potentials

The action potential in most nerves and skeletal muscle represents a transient reversal of the resting membrane potential [35], caused by time- and voltage-dependent changes in membrane conductance for sodium and potassium ions as first described in the squid giant axon [36]. Hodgkin and Huxley successfully applied the voltage-clamp technique to study these conductances; rather than eliciting action potentials, they held (clamped) the membrane potential fixed and measured the resulting macroscopic currents (fig. 3), so-called because they are generated by many thousands, if not millions, of ion channels. Inward sodium currents were characterized by a rising (activating) phase, followed by a current decline (inactivation), while outward potassium currents showed no such decline (fig. 3). The shapes, activation and inactivation of these current traces changed with membrane potential. Four distinct functions have been described for the sodium channel: a transmembrane ion pathway, a selectivity filter, an activation and an inactivation mechanism. In the squid giant axon, the potassium channel functions similarly, except that it does not normally inactivate. The activation (inactivation) mechanisms have been formally described as gates that open (close) on membrane depolarization and close (open) on membrane repolarization.

The Hodgkin-Huxley formalism [36] is a detailed mathematical description of sodium and potassium currents in the squid giant axon which fits the experimental macroscopic current data well and the resulting variables are capable of reproducing action potentials mathematically. With slight modifications, the Hodgkin-Huxley formalism has also been applied in the description of ionic currents in other preparations [35]. A description that is based on the observation of macroscopic currents may provide only limited information about events at the molecular level, thus there exists considerable controversy concerning an accurate description of sodium channel function at the molecular or single channel level [52]. Therefore, the Hodgkin-Huxley formalism is used here simply to differentiate between separate actions of anaesthetics on macroscopic currents.

Grouping anaesthetics according to their physico-chemical properties

Among the inhalation anaesthetics, there are mainly two different classes: alkanes and their derivatives and ether and ether derivatives. The alkanes include cyclopropane (a cyclic alkane), chloroform (derived from the normal or n-alkane methane) and halothane (a n-ethane derivative). The ethers include diethyl ether, the methylethyl ether derivatives methoxyflurane, enflurane and isoflurane. Analysing an anaesthetic in terms of its chemical groups and parent compound gives important information as to its possible mode of action. For example, diethyl ether ($\text{CH}_3\text{—CH}_2\text{—O—CH}_2\text{—CH}_3$) consists of different chemical groups, having either purely lipophilic character (—CH_3 , $\text{—CH}_2\text{—}$) or being polar (the ether linkage —O—). Purely lipophilic compounds reside primarily in the membrane interior (e.g. cyclopropane), non-ionic polar compounds reside predominantly at interfaces (e.g. alcohols) and inhalation anaesthetics have properties somewhere in between. By comparison with purely lipophilic molecules (alkanes) and strongly polar uncharged molecules (n-alcohols), the action of diethyl ether may be rationalized as falling somewhere in between, in turn providing the basis for the preliminary characterization of the even more complex halogenated ethers, such as isoflurane and enflurane. In terms of mechanisms of action, it has proved helpful to group gaseous and volatile anaesthetics according to their physico-chemical properties and distinguish between three different groups: (i) purely lipophilic, (ii) uncharged strongly polar, and (iii) inhalation anaesthetic. Haydon and his subsequent co-workers later subdivided the uncharged polar group by including carboxylic esters and ketones and added the group of ionic and ionizable compounds (for a review see [15]).

ANAESTHETIC ACTIONS ON SODIUM AND POTASSIUM CHANNELS

Anaesthetic effects on voltage-gated sodium channels

Distinct spectra of anaesthetic action. All anaesthetics that have been investigated so far have depressant effects on sodium channels [7, 15, 55, 59]. Figure 4 shows the effects of typical representatives of the three groups of gaseous and volatile anaesthetics discussed above. The anaesthetic concentrations are chosen such that the peak sodium currents are approximately half-suppressed.

When the actions of the anaesthetics are dissected in terms of the Hodgkin-Huxley formalism (fig. 4) it is clear that, in general, and depending on the concentration, anaesthetics affect all five Hodgkin-Huxley variables of the sodium current. The currents in figure 4 are reconstructed mathematically in such a way that they show the influence of the anaesthetic changes on a single Hodgkin-Huxley variable of the sodium current. Clearly there are instances where the anaesthetic effect on a single variable leads to current increase rather than current suppression. Therefore, the total inhibitory

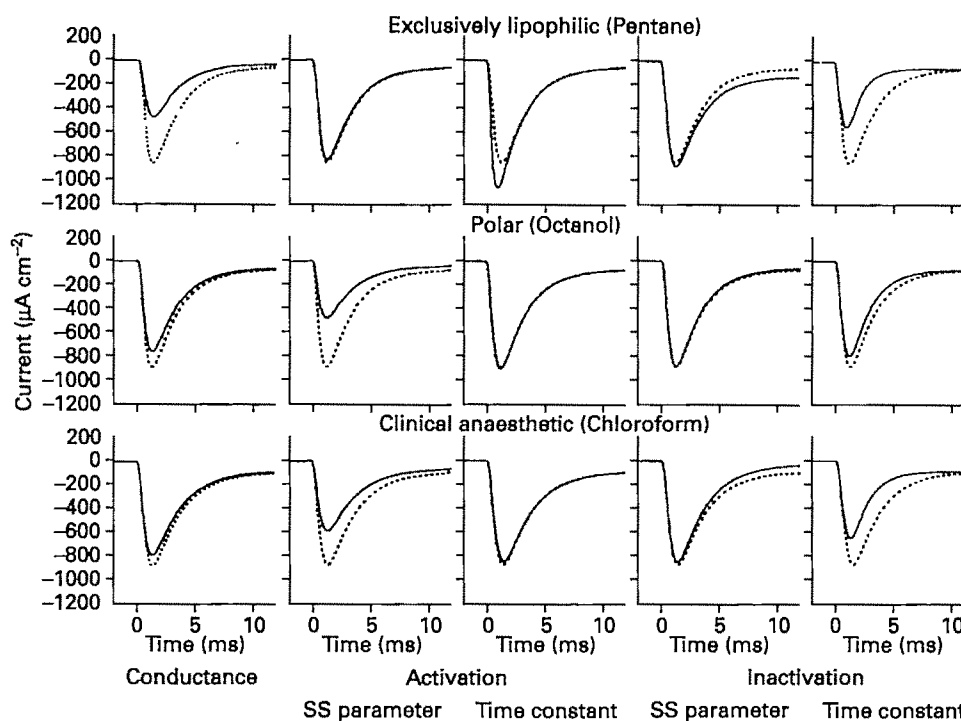


FIG. 4. Based on Hodgkin-Huxley formalism, the effects of three groups of anaesthetics on sodium currents are dissected. A set of five Hodgkin-Huxley variables each were obtained for the control (dashed line) and drug-suppressed currents by fitting the Hodgkin-Huxley equations to the experimental data [29–31]. The Hodgkin-Huxley variables of the control currents are then replaced, only one in each panel, as indicated on the abscissa, with the corresponding variable of the drug-suppressed current. The remaining four variables in each panel are those describing the control current. The resulting theoretical current (continuous line) is shown. SS = Steady state.

anaesthetic responses of sodium currents (fig. 4) results from the integration of separate inhibitory and excitatory anaesthetic actions on distinct sodium channel functions. Any one anaesthetic class suppresses sodium currents by more than one mechanism, while different classes of anaesthetics have distinct spectra of anaesthetic actions.

Purely lipophilic volatile substances. Cyclopropane [10] is an example of a purely lipophilic substance. This hydrocarbon was characterized in voltage-clamp experiments together with other cyclic alkanes, n-alkanes and carbon tetrachloride [29]. The molar concentrations of these hydrocarbons required to produce current suppression of 50% varied widely, but correlated well with lipid solubility [29], suggesting that the sites of action are essentially lipophilic.

Because of their lipophilic nature, hydrocarbons are expected to adsorb predominantly into the centre of the chain region of the bilayer, causing the membrane to thicken. This has been shown to be the case in lipid bilayer experiments and attempts have been made to demonstrate a similar effect in the squid giant axon [29]. Because of its size and simple geometry, the squid giant axon membrane, of all the biological membranes studied, is probably the most amenable to this analysis using electrical capacity measurements. Yet, even in this simple preparation, conclusions could be reached, not by direct proof, but only by consistency arguments, since the exact composition of the squid giant axon was unknown and geometrical correction factors caused by other membranes lying in front of the axolemma membrane were uncertain [30]. In so far as the nerve

membrane capacities at 100 kHz were determined by the thickness of the bilayer regions of the membrane, the results are consistent with thickening of the membrane by the hydrocarbons. A direct correlation between structural changes (evaluated via simultaneous electrical capacity measurements) and functional changes on membrane currents by the hydrocarbons was observed [26, 27, 29].

A thickening of the axolemma membrane by hydrocarbons could be expected to affect sodium channel function in several ways. The electric field (E) within the membrane may be altered as a result of the thickness (d) increase, since the electric field $E = V/d$, where V is membrane potential. Because the electric field is the physical quantity which operates on the gates of the sodium channel when the membrane potential is changed, as a consequence, the time courses and equilibrium values of the electric field-dependent variables of the Hodgkin-Huxley equations may also change [26]. In addition, and together with simultaneous membrane tension increase, as described in lipid bilayers [34], the thickness increase may subject the channels to stresses which could reduce the maximum conductance $\bar{g}_{Na}$. Inspection of the anaesthetic effects on the Hodgkin-Huxley variables follows.

All the hydrocarbons caused reduction in the voltage-independent conductance, $\bar{g}_{Na}$. This conductance is the product of the number of channels in the membrane, their availability and their single channel conductance. Haydon and co-workers, in proposing the thickness-tension hypothesis [24, 25] argued that, if a channel is normally in equilibrium with the surrounding membrane, a thickness change

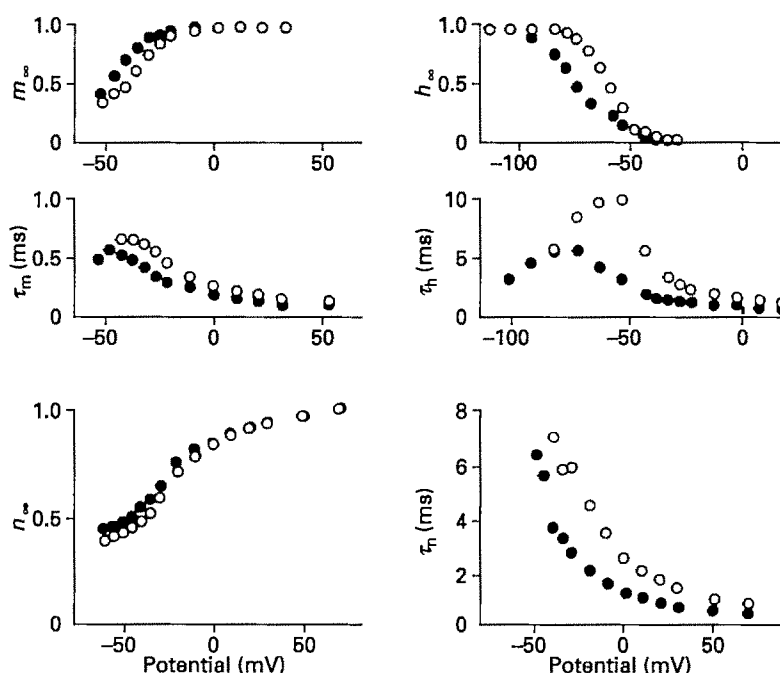


FIG. 5. Influence of n-pentane $275 \mu\text{mol litre}^{-1}$ on variables of the Hodgkin-Huxley formalism (fig. 3) for the sodium and potassium currents (●). ○ = Control values. All parameters are functions of membrane potential.

in the lipid is almost certain to stress the channel, destabilize it, and make it less available for conduction. A direct example of this mechanism was provided by the experiments with gramicidin in lipid bilayer membranes [34]. The antibiotic gramicidin behaves as a cationic ion channel that is not gated by membrane potential. It has been studied extensively in artificial membrane systems using the planar lipid bilayer technique. When lipid bilayers were formed from the same lipid, but using different chain length alkanes, similar additions of gramicidin to the system produced widely different levels of conductance. A mechanism was proposed and a theory developed in terms of mechanical stresses that are set up in the membrane that pull the gramicidin dimer apart in the middle. Essentially quantitative agreement between theory and experiment was found [34], constituting possibly the first and only quantitative example of a specific molecular mechanism by which anaesthetics reduce electrical excitability that has been verified by experiment.

If the hydrocarbon is assumed to thicken the membrane, as the electrical capacity results suggest, then the increase in thickness of the non-polar part of the membrane would change the electric field experienced by electric charges or dipoles in this region (responsible for operating the channel gates), even though the actual membrane potential remained constant. This should lead to both a shift and slope change in the voltage dependence of steady state activation and inactivation variables, as has indeed been observed (fig. 5).

However, while the correlation between electrical capacity (relating to thickness) changes and observed shifts in steady state inactivation was excellent, the slope of the curve was larger than expected [29]. This led to speculation that the average lipid composition of the membrane (as would be reflected in the electrical capacity measurement) differed from

the composition of the lipids in the immediate vicinity of the sodium channel. The shifts in the steady state activation curves were consistently less negative than in the inactivation curves. This suggested that, although the activation process was influenced by the same mechanism (e.g. membrane thickening) as the inactivation process, there was an additional effect superimposed which tended to move the activation curve in the opposite direction. When considering the physical origin of this effect for the hydrocarbons alone, there remained at least two possibilities to explain the additional mechanism; either a change in protein confirmation or an asymmetric change in the surface potential [29].

Lipophilic anaesthetics affect time constants also. For the study of mechanisms, it is necessary to separate these shift-induced changes as discussed above (fig. 5) from those caused by other effects. This can be done by considering the effect of a particular anaesthetic on the peak values of the activation and inactivation time constants. When this is done, it becomes clear that a decrease in the peak values of activation and inactivation time constants is found for most of the anaesthetics examined. A tempting explanation is that membrane fluidity increases in the presence of the anaesthetics and that the movements of proteins becomes more rapid. There is, however, relatively little direct evidence for this in the squid giant axon.

In summary, the effects of purely lipophilic substances, including cyclopropane, on the variables of the Hodgkin-Huxley equations suggest that suppression of the sodium current by these substances originates from several different effects. None of these appears to be highly specific. They may be tentatively explained in terms of adsorption of the hydrocarbons into the lipid regions of the membrane and the consequent perturbations of the sodium channel. Both the shifts in the steady state variables

and the loss of maximal conductance appeared to be accounted for by thickening of the bilayer parts of the membrane, which has two effects: change of the internal electric field after the thickening and affecting the steady state variables, and a thickness-tension mechanism reducing maximal conductance. The reduction in time constants may result from increased "fluidity" which small hydrocarbons are expected to have produced in the chain region of the membrane lipid.

Alcohols and other polar volatile substances. Alcohols do not make good clinical anaesthetics. They and other surface active substances differ from hydrocarbons in that, as a consequence of their polar groups, (hydroxyl groups for alcohols), they have only one end of the molecule localized in the bilayer interface. In contrast to hydrocarbons, for most polar substances there is little evidence of membrane thickening. Alcohols do not thicken "solvent-free" lipid bilayers [12], electrical capacity changes in squid giant axons are small and so are changes in the voltage-independent conductance, $\bar{g}_{Na}$, at anaesthetic concentrations where sodium currents are half-suppressed [30]. However, there are exceptions, such as methyl mono-octanoate, which produces a comparatively large reduction in $\bar{g}_{Na}$, suggesting perhaps a local thickening, as discussed below.

The polar substances examined in voltage-clamp experiments included the series of n-alkanols, from pentanol to decanol, and several neutral octanol derivatives with varying headgroup sizes, such as n-octyl (oxyethylene)₃ alcohol, methyl n-octanoate, glycerol 1-mono-octanoate and dioctanoyl phosphatidylcholine [30]. The alcohols produced very small effects on the steady-state inactivation at the concentrations at which sodium currents were half-suppressed (fig. 4). This is consistent with the evidence from lipid bilayer experiments and electrical capacity measurements on axons, that little or no thickness changes occur. Again, methyl mono-octanoate was an exception, leading to substantial shifts of the inactivation curve in the hyperpolarizing (negative) direction and reduction of its slope. A possible explanation for this [30] is that one or both oxygen atoms in the ester group interact with the sodium channel protein via a group embedded in the interior of the membrane. A local membrane thickening would result that could not be detected readily by other means, such as electrical capacity measurements. Therefore, it now appears necessary to invoke a site of action in or on the channel itself, in addition to interactions with the adjacent lipids.

All the polar substances examined produced a shift of the steady state activation curve in the depolarizing (positive) direction, opposite to the one observed for lipophilic substances. Following the reasoning for the hydrocarbons, the absence of membrane thickening by polar substances would cause the second mechanism that produced a depolarizing (positive) shift in the activation curve to dominate. If changes in the steady state properties of the membrane are to be invoked, dipole potentials at the surface rather than surface tension are possible candidates [30]. Benzyl alcohol has been demonstrated to modify dipole potentials in lipid bilayer

membranes [53]. However, at present there are no compelling reasons to rule out dipole potentials originating from the sodium channel protein itself. Allowing for shifts along the voltage axis, the substances within this group decreased the time constants of activation and inactivation.

In conclusion, alcohols and surface active substances also suppress sodium currents by a number of effects. They appear to act in a lipophilic environment, such as the membrane lipid, but a non-polar region of a protein cannot be ruled out as a possible site of action. While several effects may be explained by a non-specific mechanism of action, there appears to exist at least one much more specific interaction, involving a site of action in or on the channel itself.

Inhalation anaesthetics. The inhalation anaesthetics examined in this series included isoflurane, enflurane, methoxyflurane, diethyl ether, halothane and chloroform [31]. These are intermediate to hydrocarbons and alcohols in physico-chemical properties. The dielectric constants are greater than for hydrocarbons, resulting in a lesser tendency to adsorb into the lipophilic interior regions of the membrane. Yet they are also less surface active than the alcohols because they have only weak hydrogen bonding groups by which they may interact with polar molecules. Consequently, they should be less strongly localized at the membrane surface.

Suppression of voltage-independent conductance, $\bar{g}_{Na}$, is similar for the different anaesthetics and it is small in relation to suppression of the peak inward current. It resembles the suppression produced by the alcohols and other surface active substances, much more than that for hydrocarbons (fig. 4).

At the concentrations which half-suppressed the peak sodium current, the shifts in the steady state inactivation curves along the voltage axis ranged from very small values, which were scarcely different from the results for alcohols, to values which were much larger and more than half the corresponding shifts for the hydrocarbons. When another group of 11 halogenated ethers was considered also, a structure-activity correlation emerged [66]. When hydrogens in a CH₃ or CH₂ group are replaced by halogen atoms, to yield groups such as CF₃H or CClH, the remaining hydrogen(s) become acidic and capable of forming hydrogen bonds [9]. Halogenated ethers with only one strongly acidic hydrogen, such as methoxyflurane, produced large shifts in the inactivation parameters. Substances with two acidic hydrogens, such as isoflurane and enflurane, produced only small shifts. If the large shifts reflected membrane thickening then it would appear that strongly hydrogen bonding molecules could partition into the membrane interior by forming a bond with a protein group in the non-polar part of the membrane, such that the otherwise unfavourable partitioning between aqueous and hydrocarbon environments was offset. The adverse effect of two acidic hydrogens would merely suggest that there are not two suitably placed proton acceptors in the relevant region of the membrane. Halothane appears to spoil this correlation, since it has one acidic hydrogen atom, yet it causes only a small shift.

Features which distinguish it from the halogenated ethers are a smaller molecular volume and a bromine atom on the same carbon as the acidic hydrogen. Molecular volume of the hydrophobic portion of an anaesthetic molecule appears to play a role, as will be seen in a later discussion on the effects of long chain alcohols on potassium currents.

All the steady state activation curves are shifted in the depolarizing (positive) direction. In this respect, these inhalation anaesthetics also resemble the alcohols and other surface active substances much more than the hydrocarbons. Lipid bilayer studies suggest that chloroform appears to modify the surface dipole potential [53]. This would be consistent with the suggestion made when discussing the effects of the hydrocarbons and alcohols, that an unequal effect on the surface dipole potentials at the two sides of the axon membrane may be responsible. The peak values for both activation and inactivation time constants were lowered. This decrease in time constants, allowing for shifts along the voltage axis, is an almost uniform feature for all the substances in this section and the preceding sections on hydrocarbons and alcohols.

In summary, as expected from their physico-chemical properties, the inhalation anaesthetics show effects on the Hodgkin-Huxley variables that are largely intermediate between those of the hydrocarbons and alcohols. Again, several effects appear to result from non-specific actions of these drugs. However, halogenated ethers, such as isoflurane, enflurane and methoxyflurane, appear to be capable of an additional specific interaction, possibly resulting from their abilities to form hydrogen bonds. Again, the conclusion is reached that several factors contribute to the changes produced in the sodium current.

Anaesthetic effects on voltage-gated potassium channels

Comparing different ion channels within the same membrane. If the finding of non-specific interactions as an important component in the molecular mechanisms of anaesthetic action is to be generally applicable, it should find its parallel when anaesthetic actions on other ion channels are examined. With the exception of esters, ketones and halogenated ethers, no strong reasons have emerged to invoke specific interaction between the gaseous and volatile anaesthetics with either lipids or sodium channel, but several factors normally contribute to changes in sodium current. Since the above mentioned anaesthetics interact non-specifically with sodium channels, the expectation appears natural that other membrane proteins might be affected similarly. Apart from voltage-activated sodium channels, the squid giant axon membrane also contains voltage-activated potassium channels of the delayed rectifier type. There are many other types of potassium channel [35]. The potassium channels of the delayed rectifier type are important during the decreasing phase of the action potential when the membrane potential is repolarized towards the resting potential. To compare the actions of anaesthetics on ion channels within the same membrane has the ad-

vantage that differences in anaesthetic concentration may be ruled out, as explanations for the observed differences in anaesthetic sensitivity.

There are relatively few studies on interactions of general anaesthetics with voltage-gated potassium currents of the delayed rectifier type, probably because sodium currents are usually more sensitive. However, there are reasons why such a difference in sensitivity may be expected. While voltage-gated potassium channels in squid giant axons function similar to sodium channels, they do not normally inactivate. As has been discussed in the previous section, the action of anaesthetics on voltage-independent conductance, the activation and inactivation system summate. Since the action of anaesthetics on the inactivation system has resulted generally in sodium current suppression, the absence of an inactivation system would take away a mechanism by which anaesthetics may depress the ion channel function. In order to compare non-specific anaesthetic actions on sodium and potassium currents, the effects on sodium inactivation mechanism have therefore to be isolated. Using the Hodgkin-Huxley formalism, such comparisons may be accomplished easily.

Purely lipophilic, polar and inhalation anaesthetics. As in the case of sodium currents, the observation is made again that anaesthetics affect several functions of the potassium current system, reducing the voltage-independent conductance, as well as shifting steady state activation curves and changing activation time constants (fig. 6). There are striking parallels between the sodium and potassium systems. In both current systems, at concentrations that half-suppressed sodium currents, the voltage-independent conductances were reduced by lipophilic substances by a similar amount, that is about one-third, while the group of n-alcohols produced decreases of, at most, 10%. A similar observation was made with another ion channel, the antibiotic gramicidin. The time-averaged conductance of this channel, corresponding to the membrane voltage-independent conductance of sodium and potassium channels, was also much more depressed by lipophilic compounds than by alcohols [34, 63]. As discussed before, the thickness-tension hypothesis may provide an explanation for this similar behaviour of three very different membrane ion channels. There are differences also for inhalation anaesthetics. Methoxyflurane and diethyl ether reduced voltage-independent potassium conductance more effectively. Also, at larger concentrations, the normal alcohols decreased significantly both voltage-independent potassium and sodium conductance and the suggestion was made that this effect could arise from a weaker interaction with another site, perhaps inside the channel [33].

Most of the anaesthetics examined affected steady state activation by shifting it in the depolarizing (positive) direction. Comparing these shifts with those for sodium currents showed a broad qualitative correlation, but the quantitative agreement was poor. For sodium currents it had been argued that dipole potential changes could explain these shifts [30]. If these dipole potentials originate in membrane lipids

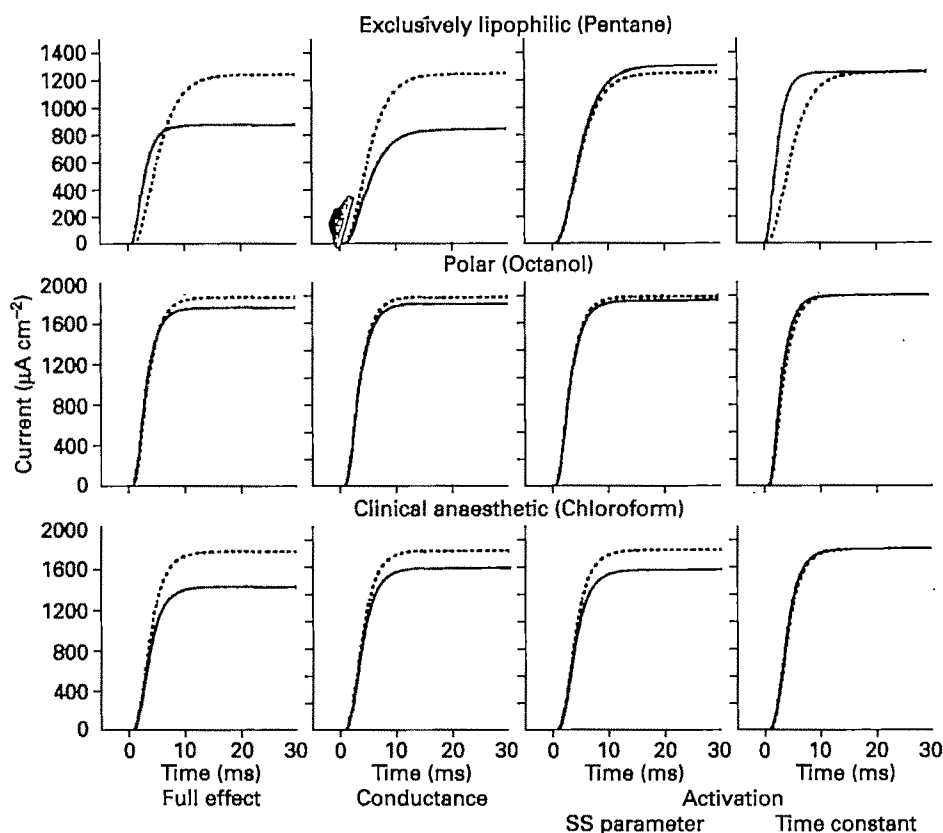


FIG. 6. Suppression of K^+ currents by typical representatives of three groups of anaesthetics (left column of panels) at concentrations comparable to those in figure 4: pentane $275 \mu\text{mol litre}^{-1}$, octanol $330 \mu\text{mol litre}^{-1}$ and chloroform $5 \text{ mmol litre}^{-1}$ at 0 mV membrane potential [33]. Based on the Hodgkin-Huxley formalism, the effects of three groups of anaesthetics on potassium currents are dissected (remaining three columns of panels). A set of three Hodgkin-Huxley variables each were obtained for the control and drug-suppressed currents by fitting the Hodgkin-Huxley equations to the experimental data [33]. The Hodgkin-Huxley variables of the control currents are then replaced, only one in each of the following panels, with the corresponding variable of the drug-suppressed current, the remaining two variables in each panel are those describing the control current. The resulting current (continuous line) is shown.

then, in order for the anaesthetics to have an effect, they would have to affect the dipoles on the extra- and intracellular membranes differently, since otherwise there would be no additional potential decrease across the membrane. If this were the case, the lipid environment of sodium and potassium channels would have to be different. The alternative would involve the dipoles attached to the membrane channel itself. The latter explanation would mean a direct interaction between anaesthetic and ion channel.

Further evidence for direct interaction with the channel protein stems from experiments with carboxylic esters [14] and halogenated ethers [66]. Most of these substances form hydrogen bonds while, in addition, the halogen atoms in substituted substances, such as isoflurane or halothane, may undergo specific interactions with chemical groups that exhibit electron donor properties [43]. The observed differences in the shifts of sodium and potassium current steady state activation could be correlated [33] in terms of electron-accepting anaesthetics shifting more potassium current activation, while anaesthetics that can donate electrons shift more sodium current activation.

As for sodium currents, changes in the activation time constants may be understood as a combination

of increased rate constants (possibly as a result of changes in membrane fluidity) together with a shift along the voltage axis.

In conclusion, the effects of a range of gaseous and volatile anaesthetics on the Hodgkin-Huxley variables suggest that depression of potassium currents, similar to depression of sodium currents, originates from more than one type of interaction. The comparison of the two current systems is consistent with the idea that, as well as interacting non-specifically with the adjacent lipid, certain anaesthetics may have an additional site of action in or on a membrane channel itself.

More than one site of action. There is further evidence for specific anaesthetic interactions. Normally, potassium currents in the squid giant axon do not inactivate, except in special circumstances [12]. However, potassium currents at larger membrane depolarizations exhibit a pronounced maximum (fig. 7) in the presence of methoxyflurane, and certain other, but not all, halogenated ethers and *n*-decanol. Although other *n*-alcohols and benzyl alcohol also cause a decrease in potassium currents, the effect is most noticeable with the longer chain alcohols, such as *n*-nonanol and *n*-decanol [33]. Mathematically, inactivation of the potassium current may be described by an inactivation term [33], analogous to the

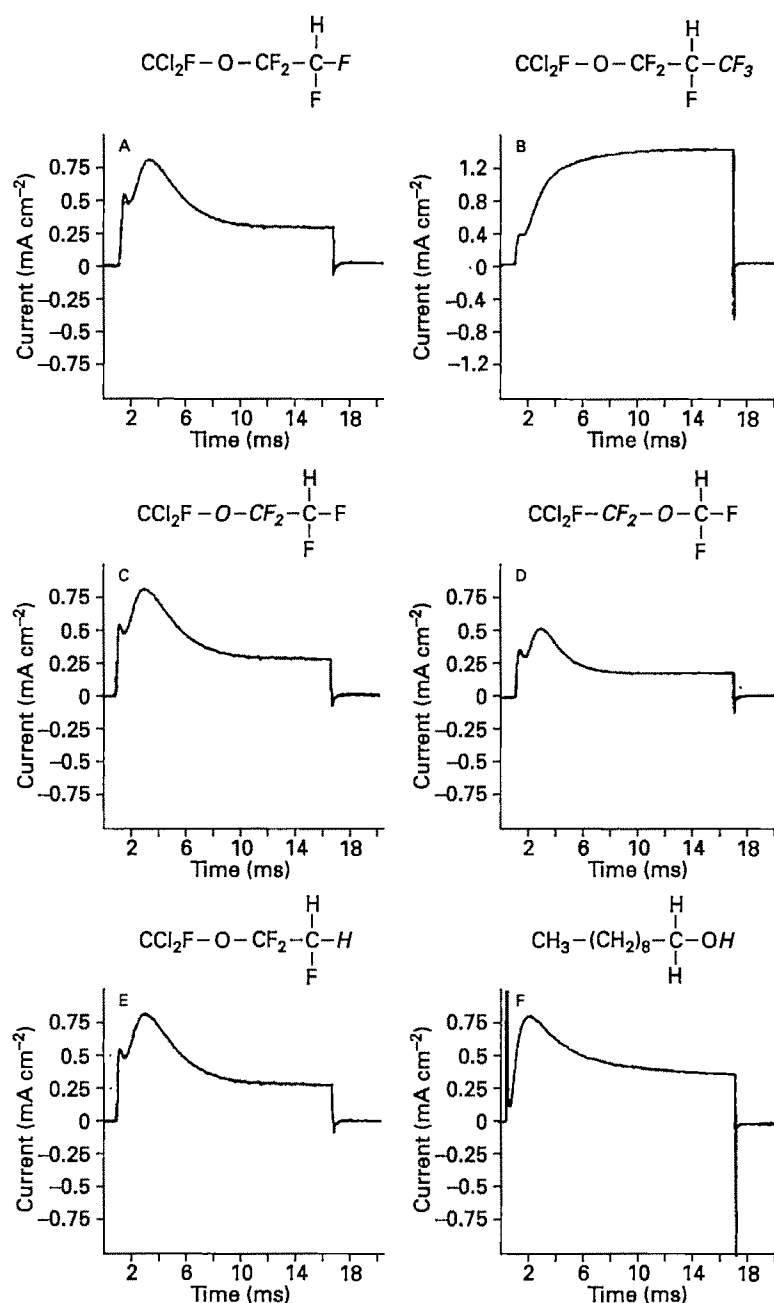


FIG. 7. Structure-function analysis of inactivation-like behaviour of potassium currents induced by different halogenated ethers and decanol [66]. Membrane potentials were depolarized to +75 mV. In the upper two panels, from left to right, only one (italics) chemical group has been changed in the halogenated ether molecule. In the bottom panels, the acidic hydrogen in the halogenated ether (left) and the alcohol decanol (right) have been changed (see text for full explanation).

one used for the description of sodium current inactivation (fig. 3). The physical basis for this apparent inactivation is not yet clear. It could be the consequence of a channel block [2, 11, 47], but it is also possible that the compounds that lead to inactivation-like behaviour in potassium currents simply accelerate a process (just as inactivation in sodium currents is generally speeded up, see fig. 4) that is latently present [12] but too slow to be obvious under normal conditions.

The presence of an acidic hydrogen atom in the halogenated ether molecule appears to be necessary but not sufficient to produce potassium current inactivation. The compound in figure 7B differs from that in figure 7A only by an additional CF_3

group that replaces a terminal fluorine atom. In contrast to lipophilic interactions, polar hydrogen bonding interactions are directed. Therefore, they may be compromised by inappropriate molecular dimensions (e.g. the above bulky CF_3 group) or competing interactions, such as the formation of hydrogen bonds by other acidic hydrogen atoms within the same substituted ether molecule. This could be the explanation as to why inactivation-like behaviour is not seen with isoflurane (two strongly acidic hydrogens), hardly present with enflurane (two strongly acidic hydrogens), but is pronounced with methoxyflurane (only one acidic hydrogen of comparable strength to the ones in isoflurane or enflurane). These findings may also be related to the

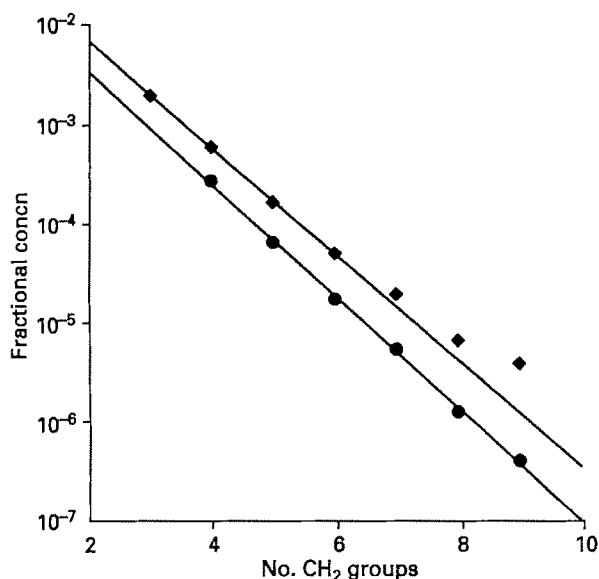


FIG. 8. Chain length dependence of n-alkanol concentrations (expressed as mole fractions) required to half-suppress peak sodium (●) or potassium (◆) current (at large depolarized membrane potentials) [33]. The slopes of the curves yield a standard free energy of adsorption per methylene (CH_2) group to the site of anaesthetic action. The values of $-3.04 \text{ kJ mol}^{-1}$ (sodium current) and $-2.91 \text{ kJ mol}^{-1}$ (potassium current, low chain length) are very similar and suggest a lipophilic environment for the adsorption site. Note the deviation from linearity in the potassium current response to longer chain alcohols.

observation that, depending upon the position of the halogen substitution, halogenated ethers may have anaesthetic or convulsive potency [58]. The halogenated ethers that produce pronounced inactivation of potassium currents share certain structural features with the longer chain alcohols. In particular, they contain a lipophilic chain, since their oxygen atoms (fig. 7C and 7D) have lost their basicity and no longer have polar but rather alkane character. Because of this, the position of this oxygen within the chain no longer matters, both derivatives in figures 7C and 7D lead to inactivation. This lipophilic chain is terminated at one end by a polar group (an acidic hydrogen, fig. 7E) with a hydrogen bonding capability of comparable strength of alcohols (the H in the $-\text{OH}$ can hydrogen bond, fig. 7F). In these, the sizes of the molecules are similar to n-nonanol and n-decanol. The sizes of the molecules relate to the strength of their lipophilic interactions, which appear to be important in explaining their mode of action. It appears that the molecular volume of the hydrophobic portion of the anaesthetic molecule is critical.

While the observation of inactivation-like behaviour strongly suggests at least two different sites of action, more evidence comes from analysis of sodium and potassium current suppression by n-alkanols (fig. 8). When anaesthetic potencies (half-suppression concentrations) are plotted against the number of methylene groups, the slopes of the resulting curve yield, under certain assumptions, the free energy of adsorption per methylene group to the site of anaesthetic action [33]. The values for the free energies (fig. 8) reveal a lipophilic site and are almost the same as for adsorption into phospholipids or an

n-dodecane-aqueous solution interface. For the potassium current, however, the magnitude of this free energy decreases for longer chain alcohols, as if they were progressively excluded from a lipophilic environment, suggesting a second site of action. The fact that no such exclusion occurs for the action of the same alcohols on sodium currents suggests that although the sodium and potassium current systems reside in the same membrane, they are surrounded by different lipids. Alternatively, these sites are on the channel proteins themselves. Whichever possibility proves correct, it appears clear that there must be more than one site of anaesthetic action.

CONCLUSION

Diversity of anaesthetic mechanisms and sites of action

Not all anaesthetics act alike. Their actions depend on their physico-chemical properties. Functionally and structurally different groups may be distinguished for small organic compounds with anaesthetic actions. Any one anaesthetic suppresses membrane ion currents by more than one action, while anaesthetics from different groups have distinct spectra of anaesthetic actions. When the different effects are summed, the suppression of sodium and potassium currents correlates with lipid solubility (fig. 9).

While the case for more than one site of anaesthetic action is strong, their locations have not been unambiguously identified. Based on consistency arguments, the lipid bilayer interior, in addition to the bilayer interface, as well as polar groups at or around the sodium channel protein, appear to be probable candidates. Even hydrophobic domains on the sodium channel protein itself cannot be excluded. Evidence from macroscopic current measurements concerning the underlying molecular mechanisms is indirect, but surface potentials, membrane thickness, surface tension, microviscosity and hydrogen bonding have been invoked; however, molecular rather than macroscopic techniques appear to be required in order to unambiguously identify anaesthetic actions on a molecular scale.

The pattern that emerged when comparing anaesthetic actions on sodium, potassium and gramicidin channels, may suggest that different anaesthetic compounds have similar lipophilic interactions with different ion channels, but that they differ in terms of additional polar interactions, for example, hydrogen bonding. However, lipophilic interactions appear to be important. As has been discussed, sufficient lipophilic volume of the anaesthetic appears to be required before certain anaesthetic actions are observed. However, it may be the polar interaction that gives an anaesthetic molecule its distinct characteristics and may lead to differentiation between different groups of anaesthetics. In this sense, isoflurane and enflurane may have become more specialized general anaesthetics than their parent compound ether, while propofol may be in some ways more closely related to relatively undifferentiated inhalation anaesthetics than to other i.v. anaesthetics.

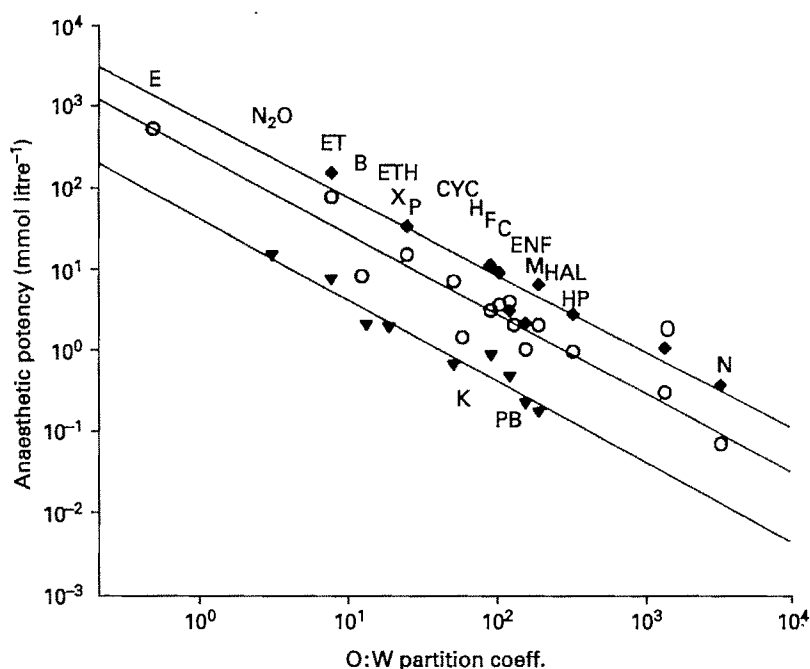


FIG. 9. Meyer-Overton correlation for human MAC equivalent (▼) (data as in fig. 2, slope -0.991) as well as Na^+ current (○) (slope -0.975) and K^+ current (◆) (slope -0.945) half-suppression [22, 33, 60]. Octanol:water (O:W) partition coefficients from Roth and Miller [56]. Note the deviation of enflurane and methoxyflurane data from the correlation for potassium current suppression. E = Ethanol; N_2O = nitrous oxide; ET = ether; B = benzyl alcohol; ETH = ethylene; X = xenon; P = pentanol; CYC = cyclopropane; H = hexanol; F = fluorene; C = chloroform; ENF = enflurane; M = methoxyflurane; HAL = halothane; HP = heptanol; O = octanol; N = nonanol; K = ketamine; PB = pentobarbitone.

Clearly, ion channels may differ in their sensitivities to different groups of anaesthetics. The comparison of anaesthetic actions on voltage-gated sodium and potassium channels discussed above has shown that in peripheral nerve, many of the gaseous and volatile anaesthetics suppress sodium currents more than potassium currents, with the group of halogenated ethers forming at least one exception (fig. 9). Thus it has become clear that in order to fully understand the molecular mechanisms of anaesthesia, the whole range of anaesthetics has to be studied and that caution should be exercised before general conclusions are drawn from examining only a few anaesthetics on a single type of preparation.

Potential correlations between molecular and clinical or physiological effects

It appears that each individual species of membrane ion channel has its own characteristic set of responses when exposed to a range of anaesthetics. Like fingerprints, these responses should be useful in identifying ion channels and possibly neuronal circuits that play an important role in a clinical or physiological reaction during anaesthesia. There are several examples of potential correspondences. Artusio observed that the clinical excitement phase was seen only during diethyl ether induction but not when patients returned to consciousness [4]; in the squid giant axon, excitability often increased during induction and then returned to normal because different anaesthetic effects appeared to reach their steady state values more rapidly than others [27, 28]. Electroencephalographic studies of seizure activity in dogs during anaesthesia reported seizure-like EEG tracings with ether derivatives but not with

non-ethers [37], correlating with the absence or presence of spontaneous action potentials in the squid giant axon when exposed to the same anaesthetic [66]. Halogenated ethers may be qualitatively different in their actions on sodium as well as on potassium channels; the number and position of the acidic hydrogens these halogenated ethers possess appear to be important in determining their actions and may also be the explanation for why some halogenated ethers act as anaesthetics, but others act as convulsants [58]. The importance of hydrogen bonding in the anaesthetic response is also consistent with a recent finding of stereospecificity in the action of optical isoflurane isomers on molluscan potassium conductance and nicotinic acetylcholine receptor [21]. The spontaneous firing of action potentials observed when exposing peripheral nerve to certain hydrocarbons [28] may be connected with cardiac arrhythmias observed with cyclopropane. Finally, the temporal dispersion increase in heart rate in the presence of local anaesthetics observed by Kasten [40] may be related to the variability increase in steady state activation of sodium channels caused by a barbiturate [23].

In order to elucidate the molecular mechanisms of anaesthetic interactions with membrane proteins, at least three different types of structure-function studies are required in which the following components are systematically varied: (i) the anaesthetic molecule, (ii) the membrane protein, and (iii) the lipid bilayer composition. Studies of the first two types have already been carried out successfully using conventional macroscopic electrophysiological techniques. However, rather than varying membrane protein species, which has been done previously and

represents a large change, it will be of great interest to systematically alter the structure of a given protein using molecular cloning techniques [41] and monitor the resulting functional changes. Electrophysiological studies of this and the third type require a different approach, one of which will be discussed in another article in this volume.

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STRUCTURE AND FUNCTION OF CALCIUM CHANNELS AND THE ACTIONS OF ANAESTHETICS

D. A. TERRAR

Specific channels for the flux of calcium ions across cell membranes are among the earliest ion channels to have evolved and are present in all animal phyla, including protozoa. Calcium channels are present in most cell types in the mammalian body and play a wide variety of functional roles in cellular processes, including control of transmitter and hormone release, contraction of muscle (cardiac, smooth and skeletal), fertilization and the control of calcium/calmodulin-dependent protein kinases. This article reviews the structure and function of various types of calcium channels and discusses the possibility that calcium channels may be targets for the actions of anaesthetics. It is clear that anaesthetics act on calcium channels in some tissues to cause unwanted or harmful effects (for example leading to reduced cardiac output as a consequence, at least in part, of altered calcium fluxes in cardiac ventricular muscle cells) and it is possible that actions of anaesthetics on calcium channels in neurones may contribute to loss of consciousness. There have been other recent reviews of various aspects of calcium channels [6, 20, 39, 61].

WHAT IS A CALCIUM CHANNEL?

A calcium channel may be viewed as any channel which allows calcium ion flux across cell membranes, but such a definition would include for example end-plate channels at skeletal neuromuscular junctions which when open, allow calcium ions to enter, but which have a much greater permeability to sodium and potassium ions. In this review, calcium channels will include only those that show ability to discriminate in favour of calcium ions, often with selectivity ratios relative to other ions of more than 1000. This ability to discriminate is functionally important in allowing selective calcium influx, since sodium ions are present in plasma at concentrations approximately 100 times greater than that of calcium. Under special circumstances (e.g. large depolarizations), significant amounts of potassium may flow out of cells across calcium channels, but cytosolic concentration of potassium is approximately 10^6 times greater than that of free calcium. The ability of

calcium channels to favour permeation by calcium appears all the more remarkable when it is considered that the unhydrated radius of a calcium ion (0.099 nm) is not very different from that of sodium (0.095 nm) or potassium (0.133 nm) ions and that ions as large as tetramethylammonium (approximately 0.6 nm) may pass through under certain conditions [30]. Sodium ions permeate calcium channels much more readily if the calcium concentration is small (less than $1 \mu\text{mol litre}^{-1}$) and it is thought that selective binding of calcium ions to sites within a channel leads to exclusion of, for example sodium ions [2, 19]. This selective binding of calcium to perhaps two sites in the channel is therefore vital for the selectivity of calcium channels. As will be discussed later, the channels appear to be membrane-spanning proteins made up of several subunits.

CLASSIFICATION OF CALCIUM CHANNELS

The opening of calcium channels to allow calcium flux (often referred to as “gating”) is in many cases controlled by the electric field across the membrane. These “voltage-gated calcium channels” may be distinguished from others where the opening is brought about by binding of a transmitter or hormone (“ligand-gated calcium channels”). Early observations in invertebrate preparations first raised the possibility that there may be more than one voltage-gated pathway for calcium entry [15]. The simplest interpretation of these experiments was that there are distinct calcium channel types with different voltage thresholds for activation and different kinetics for opening and closing. Similar observations in cardiac muscle cells led to the suggestion of L and T type calcium currents [5]. Work in neurones also suggested multiple channel types [28].

At least three different methods may be used to classify calcium channels. The experiments outlined above are examples of the biophysical approach. Biophysical characteristics used to characterize channel currents include the range of membrane potentials over which channels are activated (“threshold voltage”), the kinetics of opening and closing (activation, inactivation and deactivation) and the conductance and lifetime of individual channels. Another means of classification is based on the ability of drugs and toxins to discriminate

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between pathways. A third possibility is to use molecular biological characteristics. The channels are made up of protein subunits and discrimination between calcium channel types may be made at the level of amino acid composition of particular subunits or in the number and character of subunits making up the channel.

All methods of classification have advantages and disadvantages and there are areas of disagreement and lack of information. It appears likely that broad groups defined on biophysical and pharmacological grounds may have to be subdivided because of tissue and species differences. It should be borne in mind also that neurotransmitters and hormones may alter the channel characteristics which have been used for characterization, making the picture even more complex. Molecular biological methods may eventually provide the most unambiguous separation of channel types, but the process of fitting together this new information with previous work has a long way to go.

Biophysical methods

Voltage-gated calcium channels are thought to be proteins which span the whole width of the cell membrane and which sense the electrical field across it, allowing control of opening and closing (processes which may be seen as voltage-dependent isomerizations corresponding to the adoption of permeant or impermeant molecular conformations). Different types of channel may be activated (and inactivated or deactivated) by sensing different changes in electric field, for example if positive charges were arranged differently in various calcium channel proteins.

These possibilities have been studied in single cells in which the total current across the cell membrane ("whole cell current") is recorded in response to changes in membrane potential (by stepping the membrane potential at which the cell is voltage clamped from a steady "holding" level to a series of test potentials). It was found that some calcium current was activated by small depolarizations from the resting membrane potential ("low threshold" or low-voltage-activated) while there appeared to be a separate phase of activation of calcium current for large depolarizations ("high threshold" or high-voltage-activated) [5, 13, 15, 40]. Also, the time to peak and rate of decay of the currents appeared to differ for the low threshold (fast to peak with rapid decay) and high threshold (slower time to peak and slower decay) pathways. Similar observations have been made in a variety of cell types and, as mentioned above, led to the designations T for transient and L for long lasting [5, 13, 32, 40, 61]. While this separation appears justified, the terms themselves should be treated with caution since L type currents in cardiac muscle, while slower than T type currents in the same tissue, are considerably faster than L type currents in smooth muscle or in many neurones; for example compare dihydropyridine-sensitive calcium currents in cardiac muscle [36] with currents in neurones [13].

Studies using patch-clamp techniques to reveal activity of single channels showed that clear differences could be detected between channels thought to correspond to T and L type currents [40]. For T

channels the average open times were short and the conductance of individual channels small, in contrast to the L channels which were relatively large and long lasting. In some tissues, particularly neurones, a third category of channels was discovered with characteristics intermediate between those of T and L [24, 40, 44]. These were referred to as N, initially because they were neither T nor L, but the terminology appeared more justified when it became clear that N channels are particularly important in neurones. One point, in relation to single channel studies, is that almost all the work has been carried out using barium in place of calcium as a charge carrier to make the measurements less difficult (partly since the duration of openings of the calcium/barium channels is increased and partly to block potassium channels).

Another calcium current which does not appear to fit into the above categories has recently been described in neurones and is designated P because it was first described in Purkinje cells [27, 50]. It is thought to occur in the preterminal fibre of the squid giant synapse also [27]. The channel carrying this current appears to be activated by intermediate or high voltages and to show slow inactivation. The channel conductance is approximately 10–12 pS.

Pharmacological methods

Additional support for L, T, N and P channels is provided by experiments with drugs and toxins, although it should be emphasized that there are indications, even from observations described so far, that these categories may themselves require subdivision. Perhaps the most well known and widely used blocking substances at calcium channels are the dihydropyridines, such as nifedipine. These drugs selectively block L channels, with little or no effect at T, N or P channels [5, 13, 32]. The dihydropyridine Bay K 8644, which acts as an "agonist" to selectively enhance the opening of L type calcium channels, has proved even more useful in separating channel types [38, 40]. There is less ability to discriminate in the case of the currently available substances acting on T channels: amiloride [57] and tetramethrin [62] have been used, and Ni shows a small preference for T compared with L channels (in contrast to Cd which shows opposite, although minor selectivity). Another useful substance for investigation of calcium channels is ω -conotoxin, from the marine mollusc *Conus geographus*, which appears to act on N channels, without effects on T or P channels, although L channels are reduced in some preparations [31, 41]. For P channels, FTX toxin from the funnel-web spider is thought to act as a selective blocker [27].

Data supporting classification of calcium channels on a biophysical and pharmacological basis are summarized in table I.

Molecular biological methods

The structure and functions of sodium, potassium and chloride channels are discussed elsewhere in this issue and it is clear that different types of channels, including the calcium channels considered here, share many features in common. The first calcium channel to be investigated in detail was the

TABLE I. Classification of calcium channels based on biophysical and pharmacological characteristics. †Measured with barium as a charge carrier. DHP = Dihydropyridine; F-toxin = funnel web spider (Agelenopsis) toxin

	Calcium channels			
	L	N	T	P
Activation range	+ve to -10 mV	+ve to -20 mV	+ve to -70 mV	+ve to -40 mV
Rate of inactivation	Slow	Fast(?)	Fast	Slow
Single channel conductance (pS)†	25	15	8	12
DHP sensitivity (antagonist and agonist)	Sensitive	Resistant	Resistant	Resistant
Conotoxin sensitivity	Weak(?)	Strong	Weak	Weak
F-toxin sensitivity	Weak	Weak	Weak	Strong

dihydropyridine binding site in skeletal muscle [8, 9]. The transverse tubular membranes of skeletal muscle provide a rich source of this protein which is thought to play an important role in excitation-contraction coupling. The extent of calcium flux through this protein when it is in place in the transverse tubules is thought to be limited, although it may function as a calcium channel (of the L type since it is sensitive to dihydropyridines) in artificial membranes [9, 20, 51].

The protein is thought to comprise five subunits ($\alpha 1$, $\alpha 2$, β , γ , δ) [8]. The amino acid sequence of the $\alpha 1$ subunit was the first to be determined by cloning and sequence analysis of DNA complementary to its mRNA [56]. The $\alpha 1$ subunit was found to comprise 1873 amino acids. This component shows many of the important features of the L type calcium channel (ability to bind dihydropyridines, ability to carry

calcium current in cells transfected with complementary DNA for this subunit but lacking the other subunits, phosphorylation by cAMP-dependent protein kinase). It also shows striking sequence homology with other cation channels, including the sodium channel.

The folding of the $\alpha 1$ subunit in the membrane was suggested to be as shown in figure 1, based on the predicted hydrophobicity of different sequences [56]. There are four sets of six α helical transmembrane sequences. In each of these sets of six, the fourth (S4) region contains many positive amino acids (consistent with a role as a voltage sensor for this voltage-gated channel). The structure of the channel is considered in more detail below.

Similar molecular biological methods have been used to determine the amino acid sequence of the $\alpha 1$ subunit of the cardiac L type calcium channel, which has been found to be 2171 amino acids in length and to show 66% sequence homology with the corresponding skeletal muscle subunit [35]. Thus these techniques show that the L type channels from the two sources are similar but not identical. More cDNA clones with sequence homology to the skeletal and cardiac muscle L type $\alpha 1$ subunit have been isolated from rat brain [53]. These clones have been classified into four groups: A, B, C and D. It has been suggested (on the basis of Southern blot analysis and DNA sequencing) that each group corresponds to a distinct gene or gene family. Another research group [43] used a different nomenclature, with classes 1-5. In this terminology, class 1 corresponds to skeletal muscle L type $\alpha 1$, class 2 (Snutch class C) to cardiac L type $\alpha 1$, class 3 (Snutch class D) to neuroendocrine L type $\alpha 1$, class 4 (Snutch class A) to a neuronal channel type (possibly P) and class 5 (Snutch class B) to another unknown neuronal channel type [61]. Note that the $\alpha 1$ subunit of L type channels may well show

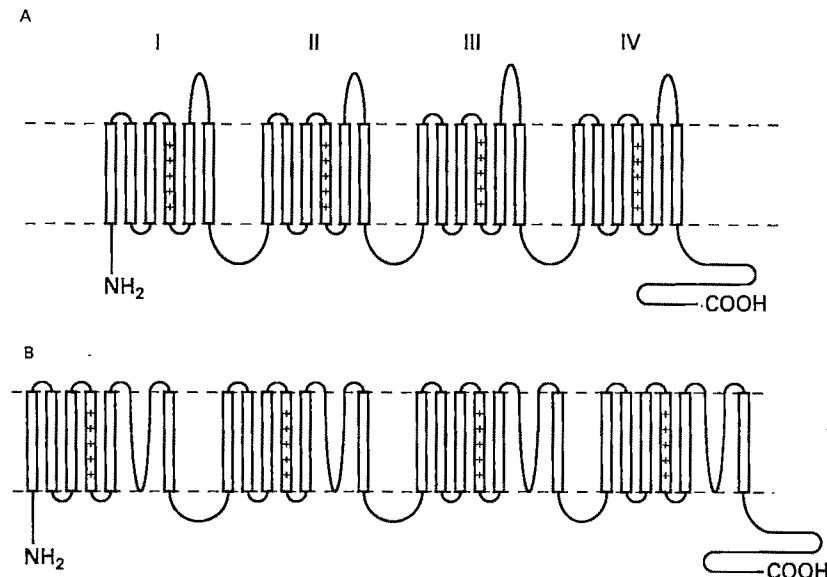


FIG. 1. Proposed folding of the $\alpha 1$ subunit of L type calcium channels in the surface membrane. There are four repeated sets of six transmembrane α helices. The fourth in each set contains a number of positive charges and may act as the sensor for voltage gating. In A, the β chains between the fifth and sixth α helix in each of the four sets are shown in the extracellular medium, whereas in B this region is shown folding into the membrane where it is postulated to form part of the lining of the pore (see text).

structural similarities with components of N, T and P channels. Indeed, recent evidence shows that ω -conotoxin-sensitive N type channels contain an $\alpha 1$ -like subunit [1]. Thus molecular biological approaches continue to yield exciting new information on the structures of different calcium channels and classification on this basis may eventually supercede classification on biophysical or pharmacological grounds.

STRUCTURE OF CALCIUM CHANNEL OF SURFACE MEMBRANE

The peptide chain of the $\alpha 1$ subunit of the calcium channel is thought to contain four repeats, each containing six α helical membrane-spanning sequences (fig. 1) and that the four sets are arranged in a ring in the membrane to form the pore of the channel, as shown in a top view (looking down on the channel) in figure 2. Recent experiments have led to the suggestion that the β strands (P region) between the fifth and sixth membrane-spanning α helices, which were originally placed outside the membrane, are in fact themselves within it, and the four similar regions on each of the four sets may form part of the lining of the pore (see fig. 1B). The evidence for this is most convincing for potassium channels [16, 64, 65], although it has been argued that the same structure is likely for cation channels, which includes calcium channels [54]. Recent evidence supports this suggestion and also shows that single mutations may confer calcium channel characteristics on the sodium channel protein [17]. What are the functions of the remaining subunits (fig. 3)? The presence of $\alpha 2$ and δ subunits has been found to increase the amount of $\alpha 1$ incorporated into the membrane and thus to increase calcium current [35]. The $\alpha 2$ - δ complex and γ subunits are also thought to modify the voltage-dependent gating characteristics of the channel [52]. The β subunit of cardiac muscle has also been found to increase peak currents and to accelerate activation kinetics [42]. Heterogeneity of subunits has been described (e.g. β subunits in cardiac muscle [22]) and it seems that expression of different types of subunits may have functional importance in determining precise characteristics of calcium channels.

CALCIUM CHANNELS OF INTERNAL MEMBRANES

The internal membranes of cells also contain calcium channels which are important for releasing calcium into the cytosol where calcium ions can regulate cell function. Two types are known, one of which is stimulated to release calcium in response to inositol 1,4,5-triphosphate (IP₃) and another which is very sensitive to a plant alkaloid, ryanodine. The ryanodine receptor in sarcoplasmic reticulum membranes is thought to function differently in skeletal and cardiac muscles: in both cases, calcium is released to control contraction, but for skeletal muscle, the dihydropyridine receptor in the surface membrane is thought to sense the voltage change during an action potential and interact with the "foot" of the ryanodine receptor to open it, whereas in cardiac

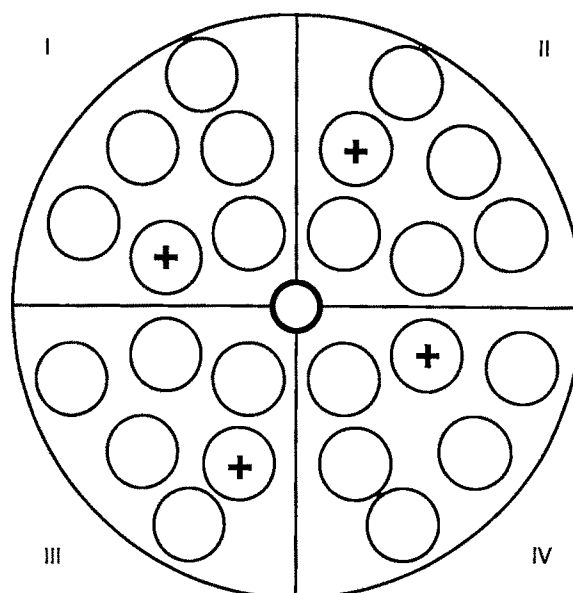


FIG. 2. Diagrammatic representation of the surface of the $\alpha 1$ subunit viewed from above, looking down from the extracellular medium. The four sets of α helices (and the folded β chains) are thought to be arranged in a ring with the pore formed at the centre.

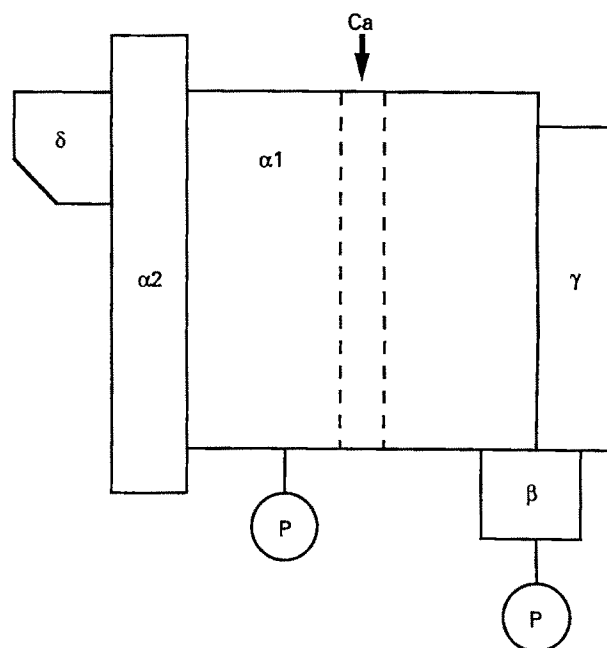


FIG. 3. Possible arrangement of subunits of the L type calcium channel. Sites for phosphorylation are shown on the $\alpha 1$ and β subunits (based on [8, 9]).

muscle calcium entry through the surface membrane is thought to be the major trigger to open the channel [9, 12, 20, 51]. The structure of the skeletal muscle ryanodine receptor has been determined and shows features in common with the surface membrane calcium channels [25, 55]. The IP₃ receptor, isolated from rat cerebellum, also shows structural similarity with other calcium channels [14].

FUNCTIONS OF CALCIUM CHANNELS

Since the actions of calcium are so widespread, it is difficult to summarize known functions of this ion. It

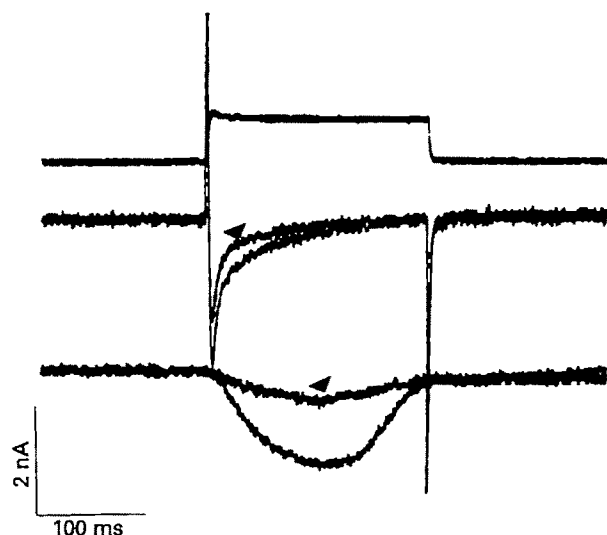


FIG. 4. Effects of isoflurane on L type calcium currents recorded from a guineapig isolated cardiac ventricular cell. Records are superimposed before and during (arrows) exposure to isoflurane $1.2 \text{ mmol litre}^{-1}$ (corresponding to 3.2% on the vaporizer under the conditions of the experiments). Upper curves: voltage clamp depolarizations from -40 to 0 mV ; middle curves: calcium current (shown in other experiments to be sensitive to nifedipine and verapamil); lower curves: measure of cell shortening (from [59]).

is perhaps because of the ubiquitous use of calcium as an intracellular messenger that all cells maintain their resting calcium at very small concentrations (approximately $100 \text{ nmol litre}^{-1}$, far from equilibrium with plasma). The importance of calcium entry to control contraction in various types of muscle and to induce release of neurotransmitters and hormones is well known. In signalling of the nervous system, an increase in cytosolic calcium which follows activation of surface membrane calcium channels often modulates the activity of other ion channels (e.g. potassium [34] and chloride [11]), which in turn may modulate neuronal excitability. Calcium channels themselves may play a role in regulating the excitability of neurones [6, 26]. Calcium also plays a role in transduction of various sensory stimuli into neuronal activity (including auditory [21] and visual [33] stimuli). Calmodulin is present in all cells and in both excitable and non-excitable cells changes in cytosolic calcium (which may arise from calcium entry through the surface membrane or from release from internal stores) are known to regulate calcium-calmodulin-dependent protein kinases which in turn regulate various aspects of cell function, often interacting with other protein kinases. Release of calcium from intracellular stores is functionally important in most cells. It is thought that the IP₃ release channel appeared early in evolution and is present in all animal phyla including protozoa [20].

ACTIONS OF ANAESTHETICS ON CALCIUM CHANNELS

General anaesthetics exert a variety of effects on the cardiovascular system, which are consistent with actions of anaesthetics on calcium channels (e.g. inhibition of SA node automaticity [7], prolongation

of A-V conduction time [4] and depression of myocardial contractility [46]). Direct evidence for this effect on dihydropyridine-sensitive L type calcium channels in cardiac ventricular muscle cells has been obtained in voltage-clamped cardiac ventricular cells for several commonly used anaesthetics, including halothane [58], isoflurane [59], enflurane [48] and propofol [47]. An example of such a record is shown in figure 4, where it can be seen that isoflurane $1.2 \text{ mmol litre}^{-1}$ (corresponding to 3.2% on the vaporizer under the conditions of the experiments) reduced the amplitude of the calcium current. The reduction of the contraction accompanying the calcium current was greater than that of the current itself. It was known from whole animal studies that different anaesthetics may have different effects on cardiac contractility (e.g. halothane appears to cause greater depression of myocardial contractility than isoflurane). At the single cell level, although reduction of calcium influx across the surface membrane is thought to contribute to a reduction in contraction, it has been suggested, from comparisons of different anaesthetics, that halogenated volatile anaesthetics, in particular, may exert additional effects, perhaps on the release channel of the sarcoplasmic reticulum [47, 59]. Indirect evidence supports this suggestion [49, 58, 59, 60].

More direct evidence for the action of halogenated anaesthetics on sarcoplasmic reticulum calcium channels has been provided by experiments in which cardiac trabeculae were chemically "skinned" with saponin, allowing calcium fluxes across the sarcoplasmic reticulum membrane to be studied. Halothane was found to stimulate calcium efflux from the sarcoplasmic reticulum [18]. Studies using fluorescent probes to monitor cytosolic calcium also support the suggestion that halothane, enflurane and isoflurane decrease the calcium content of the sarcoplasmic reticulum by increasing calcium permeability [23, 63], perhaps with additional effects on the uptake/extrusion mechanisms of the sarcoplasmic reticulum [63].

Recent evidence examining anaesthetic action on both L and T type calcium currents at the surface membrane of cardiac muscle cells shows that the activities of both channels appear to be approximately equally suppressed by halothane, isoflurane and enflurane [10].

Actions of halogenated anaesthetics, such as halothane, at the level of the calcium release channel (ryanodine receptor) in skeletal muscle are thought to underlie malignant hyperthermia [29]. The observations support the suggestion that in pigs susceptible to malignant hyperthermia, the sarcoplasmic reticulum calcium release channel is altered in such a way that the channel may be activated even at a cytosolic calcium concentration or pH that would normally be expected to maintain the channel in the closed state [29]. Similar conclusions were reached in a study of calcium release channels from patients who were susceptible to malignant hyperthermia. Of 13 channels from these patients, seven were affected by halothane, increasing the probability of the channel to change from the inactive, closed state to an open state. This effect of halothane to increase

open state probability was associated with an overall increase in channel conductance. No effects of halothane were detected on 10 of 10 channels from negatively diagnosed subjects. The observations were again taken to support the suggestion of a defect in the ryanodine-sensitive calcium release channel from patients showing malignant hyperthermia [37].

Preliminary evidence is available concerning the actions of the anaesthetics enflurane and propofol at the level of single L type calcium channels in the surface membrane, studied using the cell-attached patch-clamp technique applied to cardiac ventricular cells [47]. Openings of channels in response to depolarizing pulses were compared in the absence and presence of anaesthetics. Exposure to either enflurane or propofol applied in the solution bathing the cells caused the channels under the patch pipette to spend less time open and more time in the closed state, but did not alter the single channel conductance. It appears that the anaesthetics applied in the bath may gain access to the membrane beneath the patch pipette, although whether or not the action of the anaesthetics on the opening and closing of calcium channels is a direct effect on the channel proteins, or is a secondary consequence, for example of alterations in membrane lipids, remains to be determined. The reduced probability of single channel opening is consistent with reduction in whole cell calcium current by these anaesthetics [47].

Actions of anaesthetics on calcium currents in neurones have been reported recently. Halothane caused a dose-dependent reduction of calcium current (probably L type, HVN channels) in hippocampal CA1 neurones. In rat sensory neurones, halothane and isoflurane were found to reduce both small and large voltage-activated calcium currents, with the small voltage currents showing the greater sensitivity [55]. It was suggested that since small voltage-activated calcium currents have been postulated to be involved in the control of neuronal excitability and bursting [25], block of this pathway may explain some of the clinical actions of volatile anaesthetics. However, in clonal (GH3) pituitary cells, large voltage-activated calcium currents were found to be more sensitive to reduction by halothane

fluxes in ventricular muscle cells), while in neurones, actions on calcium channels may contribute to the desired loss of consciousness.

Our understanding of calcium channel structure and function depends on biophysical, pharmacological and molecular biological evidence. Different types of voltage-gated calcium currents have been characterized according to the range of membrane potentials over which the currents are activated and inactivated, and by the timing of these processes. Ion channels carrying the current can be studied more directly using patch-clamp techniques and are identified by their single channel conductance and by their kinetics of opening and closing. At least four types of channel (L, N, T and P in one commonly accepted scheme of nomenclature) have been characterized in this way, although subdivision of these categories appears likely. Drugs and toxins provide further support for these divisions: dihydropyridines such as nifedipine act selectively on L type channels, the mollusc-derived ω -conotoxin acts on N type channels and a component of funnel web spider toxin acts on P channels. Molecular biological approaches have shown that L type channels comprise several subunits. The $\alpha 1$ subunit is a polypeptide chain showing four repeated sections, each comprising six membrane-spanning sequences. The four repeated sections are thought to be arranged in a ring in the membrane, allowing calcium ion flux through a pore in the centre. The $\alpha 1$ subunit is thought to contain the dihydropyridine binding site. Different aspects of channel function, such as discrimination between ions or the ability to sense changes in transmembrane electric field (allowing voltage gating), are thought to be determined by specific regions in the transmembrane sequences in each of the four repeats. Other subunits of the L type channel are thought to modify the behaviour of the $\alpha 1$ subunit in the membrane (for example the β subunit appears to accelerate activation of the channel). Less is known about T, N and P channel structure, but similarities in $\alpha 1$ -like subunits appear likely from the data available. Profound changes in channel function may occur after mutations affecting individual amino acids in the polypeptide chain and recent evidence shows that sodium channels may

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EFFECTS OF VOLATILE ANAESTHETICS ON SECOND MESSENGER Ca^{2+} IN NEURONES AND NON-MUSCULAR CELLS

H. G. KRESS AND P. W. L. TAS

CYTOPLASMIC CALCIUM AND CELLULAR FUNCTION

The regulation of intracellular calcium concentration is of vital importance to every living cell. Since calcium phosphate complexes are very insoluble, phosphorylation processes, which are necessary for life, could not exist if plasma concentrations of free calcium existed within the cytoplasm. Therefore, cells had to develop powerful means to reduce the intracellular Ca^{2+} concentration. The resulting 10000 fold gradient between extracellular and cytoplasmic calcium concentration makes Ca^{2+} an ideal second messenger. Many physiological stimuli use calcium in signalling pathways, for example, transmembrane signal transduction in haemopoietic, muscle, endocrine and nerve cells, and many of these calcium-dependent processes are influenced by halogenated inhalation anaesthetics. However, evidence that volatile anaesthetics may interfere directly with the regulation of intracellular calcium and its second messenger functions is relatively sparse [59, 60].

This review focuses on calcium as a second messenger in non-muscular tissue, that is neurones, neurosecretory or glial cells, and how it is affected by volatile anaesthetics. Anaesthetic effects on calcium in heart cells is described elsewhere in this issue. Neurones use calcium transients for transmitter release and control of excitability [13, 82, 118], to integrate and communicate information. Both spatial and temporal control of intracellular calcium is necessary, and the multiple roles of Ca^{2+} in neurones are reflected by the complex regulatory mechanisms involved [5]. Ca^{2+} regulation may be different for different parts of the neurone because of the distribution of Ca^{2+} channels and Ca^{2+} binding proteins within soma, neurites and synapses. Neurotransmitter release is, for example, restricted to the synapse because of the presence of synaptic vesicles and the clustering of voltage operated Ca^{2+} channels (VOCC) in this area. This is a prerequisite for reaching Ca^{2+} concentrations large enough ($200\text{--}300\text{ }\mu\text{mol litre}^{-1}$) to stimulate neurotransmitter release [5, 65].

Some of the functions of Ca^{2+} in neuronal cells are depicted in figure 1. Cytoplasmic Ca^{2+} increases

when Ca^{2+} flows through VOCC or receptor operated Ca^{2+} channels (ROCC), or when it is released from intracellular stores in response to inositol 1,4,5 triphosphate (IP3) [11]. Increased Ca^{2+} may change the activities of certain potassium and chloride channels in the plasma membrane, which may have profound influences on neuronal excitability [75]. Increased Ca^{2+} concentrations may further activate phospholipase C (PLC) [95], which cleaves phosphatidyl inositol diphosphate (PIP2), and phospholipase A_2 which in turn cleaves fatty acids [6]. Prominent cytosolic targets for increased Ca^{2+} concentrations include protein kinase C [92, 93], calpain [135] and calmodulin [21]. The protein kinase C signalling pathway appears to play an important role in synaptic transmission [30], the perception of pain [41] and in long-term potentiation and memory [63]. The *calpains* are a family of neutral proteases that are involved in the regulation of membrane proteins and the cytoskeleton in a number of cells, including neurones [135]. *Calmodulin* is a Ca^{2+} binding protein

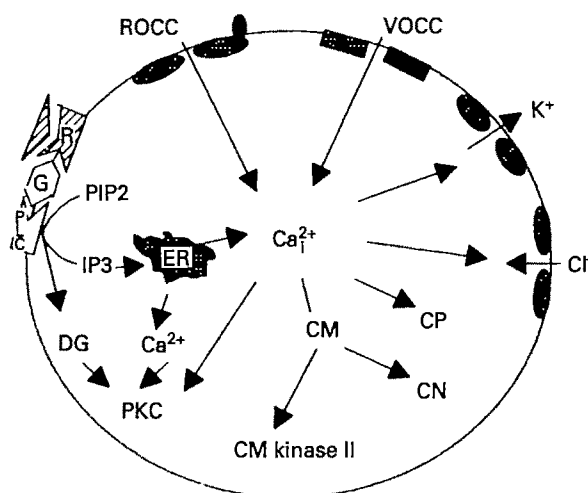


FIG. 1. Immediate targets of Ca^{2+} in neuronal cells. Cytoplasmic calcium ($[\text{Ca}^{2+}]_i$) is increased when Ca^{2+} flows through voltage (VOCC) or receptor operated (ROCC) calcium channels, or when it is released from intracellular stores in response to inositol triphosphate (IP3). Increased $[\text{Ca}^{2+}]_i$ stimulates Ca^{2+} -dependent K^+ or Cl^- channels in the membrane. In the cytosol, $[\text{Ca}^{2+}]_i$ stimulates protein kinase C (PKC), the Ca^{2+} binding protein calmodulin (CM) and the protease calpain (CP). Ca^{2+} -calmodulin in turn regulates calmodulin kinase II and calcineurin (CN) (protein phosphatase 2B).

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KEY WORDS

General anaesthetic. Ion channels: Ca^{2+} , NMDA. Mechanism of action.

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that is located in the cell bodies and dendrites of most neurones but not in axons or presynaptic terminals [89]. It activates a number of specific proteins, including calmodulin kinase II, adenylyl cyclase, cyclic nucleotide phosphodiesterase and calcineurin [21]. Ca^{2+} -calmodulin-dependent protein kinase II is concentrated particularly at post-synaptic densities [53] where it may play an important role in neuronal function. *Calcineurin* (also known as protein phosphatase 2B) is abundant in brain tissue and has been shown to be implicated in the inactivation of L type VOCC [19].

It is clear from these examples that Ca^{2+} plays a central role in the regulation of many neural and glial cell functions, and that any effect of volatile anaesthetics on intracellular Ca^{2+} or its regulation may have implications for a plethora of neuronal processes, depending on their sensitivity to Ca^{2+} .

REGULATION OF INTRACELLULAR CALCIUM

Regulation of intracellular calcium concentration is very complex and involves at least four principal mechanisms (fig. 2). Increases in cytoplasmic Ca^{2+} follow each cycle of nerve cell activity. Ca^{2+} enters the cell via several distinct types of voltage (table I) or receptor operated membrane ion channels (table II) or is released from endoplasmic reticulum [82].

The resulting calcium signals may be modulated and terminated by binding to high-affinity calcium binding proteins (calmodulin, parvalbumin, calbindin, calcineurin [37]), by sequestration of calcium in intracellular organelles, such as endoplasmic reticulum [13] or mitochondria [104], and by extrusion of calcium from the cell by membrane transport systems capable of overcoming the large transmembrane concentration gradient of calcium. Two major transport systems have been identified:

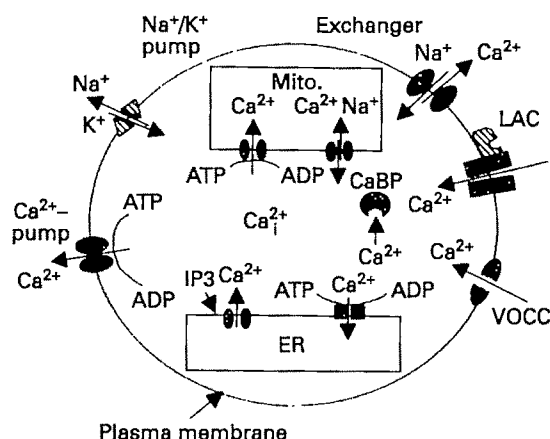


FIG. 2. Regulation of cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). Ca^{2+} flows into the cell through voltage operated (VOCC) or ligand activated (LAC) calcium channels. Ca^{2+} may be released from the endoplasmic reticulum (ER) by inositol 1,4,5 triphosphate (IP3). Ca^{2+} is removed from the cytoplasm by calcium binding proteins (CaBP) or is taken up into the ER via a Ca^{2+} pump. Ca^{2+} may be removed from the cell by a Ca^{2+} pump or a Na^{+} - Ca^{2+} exchanger. Mitochondria (Mito.) may participate transiently in Ca^{2+} homeostasis when the capacities of the other systems are exceeded. The major influx pathway of this organelle is an electrogenic Ca^{2+} uniporter, the major efflux pathway is a 2Na^{+} - Ca^{2+} exchange carrier.

the ATP-driven Ca^{2+} pump [18] and a Na^{+} - Ca^{2+} exchanger which is driven by the Na^{+} concentration gradient maintained by Na^{+} - K^{+} ATPase. The Ca^{2+} ATPase has a high affinity for Ca^{2+} but a small transport capacity and is therefore well suited for the regulation of resting concentrations of calcium. The Na^{+} - Ca^{2+} exchanger has a low affinity, but a large transport capacity for Ca^{2+} . Therefore, it is active after large increases in intracellular Ca^{2+} concentration.

EFFECTS OF VOLATILE ANAESTHETICS ON CYTOPLASMIC CALCIUM IN RESTING CELLS

Volatile anaesthetics increase cytoplasmic Ca^{2+} in a number of cells and tissues, including hepatocytes [48, 49], mouse brain synaptosomes [24], hippocampal slices [85], rat pheochromocytoma PC12 cells [125], peripheral blood mononuclear cells [55], L6 muscle cells [54] and rat glioma C6 cells [unpublished observation]. Because of the complex regulation of Ca^{2+} , in most cases it is not known which of the cellular stores or processes is responsible for the observed increase in Ca^{2+} .

In peripheral blood mononuclear cells, cytoplasmic Ca^{2+} was reduced greatly after removal of extracellular Ca^{2+} , indicating that Ca^{2+} influx may be responsible for the observed Ca^{2+} increase [55]. In L6 muscle cells, however, approximately 50% of the Ca^{2+} increase was a result of release from intracellular stores [54]. In hepatocytes, halothane has been shown to increase cytoplasmic Ca^{2+} by stimulation of phospholipase C-mediated formation of IP3 [44]. In slices of rat brain, on the other hand, halothane did not have any effect on inositol phosphate accumulation in the presence or absence of receptor agonists [8].

It has been reported also that volatile anaesthetics stimulated the release of $^{45}\text{Ca}^{2+}$ from non-mitochondrial stores in saponin permeabilized hepatocytes [49]. Halothane also inhibited the uptake of Ca^{2+} into brain mitochondria [109]. To date, no data exist on the effects of volatile anaesthetics on Ca^{2+} extrusion by Ca^{2+} ATPase or the Na^{+} - Ca^{2+} exchange, both of which are affected by other general anaesthetics [36, 136].

The anaesthetic-induced increase in cytoplasmic Ca^{2+} may activate potassium conductance that hyperpolarizes the cell, thus reducing its excitability. This hyperpolarization of vertebrate CNS neurones has been observed for many general anaesthetics [59, 60, 91]. The idea that increased Ca^{2+} may be important for anaesthesia was supported also by experiments that showed prolongation of ethanol-induced anaesthesia by intraventricular injection of Ca^{2+} [28]. In hippocampal slices, the increase in Ca^{2+} by halothane activates a GABA_A -mediated chloride current leading to depressed neuronal excitability [85].

VOLTAGE OPERATED CALCIUM CHANNELS

Physiology, biochemistry and pharmacology

Voltage operated calcium channels (VOCC) represent the principle means for Ca^{2+} to enter the cell

TABLE I. Classification of voltage operated calcium channels by electrical and pharmacological properties. LVA = Low voltage activated; HVA = high voltage activated; DHP = dihydropyridine; ω -CgTx = omega-conotoxin

	Channel type			
	T	L	N	P
Threshold of activation	LVA	HVA	HVA	HVA
DHP sensitivity	No	Yes	No	No
ω -CgTx sensitivity	No	(No)	Yes	No
Tissue	Neuronal/ Non-neuronal	Neuronal/ Non-neuronal	Neuronal	Neuronal

[9, 81, 83]. As molecular cloning has revealed, VOCC are members of the voltage operated ion channel super family which also includes Na^+ and K^+ channel proteins [83, 121, 129, 130]. Within this super family, VOCC constitute a whole family of different ionophores that, until recently, were classified mainly according to their electrophysiological and pharmacological characteristics [94, 121, 130] (table I). Based on the level of membrane depolarization required for activation, at least two distinct classes of VOCC coexist in many vertebral cells (ranging from fibroblasts to peripheral and central neurones): low voltage activated (LVA) and high voltage activated (HVA) Ca^{2+} channels [94, 105, 121, 130].

LVA, or T (for "transient") type Ca^{2+} channels may be distinguished clearly from HVA channels by the low-voltage threshold of activation and the transient opening of LVA as a result of rapid and purely potential-dependent inactivation [94, 121, 129, 130]. Thus in excitable cells, variation of neuronal rest potential affects the activity of T type VOCC, which in turn affects the excitability of the cell membrane. LVA channels are thought to enhance excitability, to promote bursting of central neurones and to control spike firing patterns in a number of different neural cell types. T channels are found on central and peripheral neurones, as well as in non-neuronal tissues including cardiac and smooth muscle, fibroblasts and secretory cells [9, 130]. In general, T type VOCC are relatively resistant to dihydropyridines, such as nifedipine, or other organic Ca^{2+} channel blockers (diltiazem, verapamil). Other compounds have been reported to block T channels in neurones in an unspecific manner (e.g. diphenylhydantoin, amiloride, octanol, tetramethrin and nickel) [129, 130].

HVA calcium channels are characterized by a large threshold of activation; slow inactivation is dependent strongly on the internal Ca^{2+} concentration and their fast deactivation [9, 121, 130]. The first HVA channel that was characterized extensively was the so-called L type (for long-lasting) VOCC found in almost every cell (e.g. heart, smooth muscle, endocrine and neuronal cells). L type VOCC are defined as HVA channels sensitive to dihydropyridine agonists (Bay K 8644) and antagonists (e.g. nifedipine, nicardipine) and may thus be distin-

guished from T, N and P type VOCC, which are generally resistant to dihydropyridines (table I) [83, 114, 129].

In the late 1980s, apart from the L type current, an additional HVA Ca^{2+} current was described in chick dorsal root ganglion cells using electrophysiological and pharmacological criteria, such as inactivation kinetics, holding potential, single channel properties and sensitivity to dihydropyridines. This VOCC, that proved to be resistant to dihydropyridines, but very sensitive to omega-conotoxin GVIA (ω -CgTx), a peptide toxin derived from the piscivorous marine mollusc *Conus geographicus*, was named the N type (neither T nor L) channel [94, 98]. N type channels are distributed widely in the nervous system but do not appear to exist in muscle [7, 32, 69]. Recently, they have been found also in endocrine cells from anterior pituitary, pancreas, adrenal gland and neural cell lines, such as PC12 [114, 115]. N channels are assumed to play a major role in mediating transmitter release which is affected little by dihydropyridines [25, 26, 38, 43, 45, 56, 97, 106, 114]. This notion is supported by the subcellular distribution of N channels and the predominant localization in "active zones" of the presynaptic terminals of peripheral neurones, where they may supply Ca^{2+} for transmitter release [83, 114, 129, 130]. Their contribution in cortical neurones is likely to be substantial since ω -CgTx sites are 20 times denser than dihydropyridine sites in the brain [129]. However, their relative prominence varies between neurones of different species [45] and also from neurone to neurone within a species. For example, N type channels are the dominant Ca^{2+} entry mechanism in sensory, sympathetic and myenteric plexus neurones, but are largely absent in cerebellar Purkinje cells and possibly cerebellar granule cells also [105].

While many neurones exhibit N type (dihydropyridine resistant, ω -CgTx sensitive) and L type (dihydropyridine sensitive, largely ω -CgTx resistant) VOCC [38, 56], some also possess a significant fraction of a third HVA current, that differs insubstantially in its voltage dependence or kinetics, but is resistant to both types of channel blocker [68, 105, 121, 129] (table I). Since this novel class of HVA channels predominates in cerebellar Purkinje cells, these channels have been loosely designated P type [64], although it is not clear how many channel types actually exist in this category [42, 64, 129]. At least in cerebellar Purkinje cells, P type VOCC are blocked by a peptide toxin obtained from the venom of the funnel web spider *Agelenopsis aperta* [64, 84, 129].

Our understanding of the functions of the different subtypes of VOCC within the nervous system is limited severely. L type channels are localized at the cell body and at the base of major dendrites in hippocampal pyramidal cells and sympathetic neurones [127], whereas ω -CgTx sensitive VOCC are found in clusters that coincide with the site of synaptic contacts [106, 114, 130]. At the neuromuscular junction, ω -CgTx sensitive channels are expressed exclusively in the presynaptic area, corresponding to active zones, where fusion of synaptic

vesicles and release of transmitter is known to occur [114]. It is therefore tempting to speculate that Ca^{2+} influx through L type VOCC located at the cell bodies may be relevant to the regulation of Ca^{2+} -dependent intracellular events, such as protein phosphorylation or activation of gene expression, whereas the strategic presynaptic localization of ω -CgTx sensitive channels might be necessary for rapid generation of large Ca^{2+} transients able to trigger fast and synchronous transmitter release from the nerve terminals [26, 98]. However, L and N type VOCC are only two members of the HVA Ca^{2+} channel family. P channels and, as yet unidentified VOCC insensitive to dihydropyridines or ω -CgTx, may be of much greater importance to presynaptic transmitter release in a number of different species, neuronal cell types, or both [68, 105, 131].

Over the past two years, rapid advances in molecular biology have pointed to an even greater diversity of VOCC than is apparent from pharmacologically based functional grouping into T, L, N and P. In muscle and neuronal cells, diversity of L type VOCC occurs through (a) the expression of at least five different genes encoding distinct classes of pore-forming $\alpha 1$ subunits, (b) the production of alternatively spliced $\alpha 1$ isoforms, and (c) regulation by associated subunits ($\alpha 2/\delta$; β ; γ) [66]. These sources of channel diversification form the basis for a functional diversity among L type HVA channels, so-called tissue specificity, and may explain differences in second messenger-dependent regulation or pharmacological sensitivity to channel blockers. Recently, the primary structure of a ω -CgTx and dihydropyridine insensitive HVA Ca^{2+} channel from rabbit brain, probably a P type VOCC, was deduced by cloning and sequencing the complementary DNA [86]. Further elucidation of the molecular properties of T and N type, or additional P type VOCC is expected in the near future.

Effects of volatile anaesthetics on VOCC

In the central nervous system, suppression of inward Ca^{2+} currents may be crucial in presynaptic nerve terminals by decreasing the amount of transmitter released [100, 142], and consecutively it may underlie the depression of excitatory synaptic transmission seen with volatile anaesthetics [100]. The idea that volatile anaesthetics block synaptic transmission by suppressing inward Ca^{2+} current in nerve terminals has been supported only by very limited electrophysiological evidence. As was demonstrated in the neocortex, the application of anaesthetic concentrations of isoflurane depressed the excitability of neocortical neurones by interfering with the processes involved in synaptic excitation [27]. Similarly, the reduction in monosynaptic EPSP in the cat spinal cord *in vivo* during anaesthesia with halothane or diethyl ether has been attributed to a decrease in quanta of transmitter released per stimulus without affecting quantal size [62, 139, 140].

In slices from rat brain, the only significant effect of clinical concentrations of halothane was a decrease in the Ca^{2+} -dependent noradrenaline release in

response to K^{+} depolarization, whereas no effect was observed on transmitter uptake, binding of adrenergic agonist and antagonists, coupling to G proteins or receptor-mediated stimulation of cAMP or inositol phosphate formation [8]. Direct evidence is provided by *in vitro* investigations in rat hippocampal CA1 neurones, where 1–3% v/v halothane caused marked and reversible suppression of a slow inward Ca^{2+} current evoked by depolarizing pulses [61]. By analogy with the inhibitory effects observed in the rat pituitary cell line GH3 (see also below) [39], the peak amplitudes of the inward currents were reduced in a concentration-dependent manner and decayed more rapidly. Although no detailed analysis was performed, the experiments suggest that LVA and HVA currents were depressed indiscriminately [61]. Since it is very difficult to study the action of anaesthetics on the mechanisms of transmitter release within the CNS, neurosecretory cells, such as adrenal chromaffin cell preparations or neural cell lines have been used to overcome these difficulties [39, 57, 58, 99, 119, 138].

At first glance, the results obtained in neurosecretory cells derived from embryonic neural crest tissue appear contradictory and confusing. Depolarization with increased concentrations of potassium ions directly activated VOCC in these cells. Investigating the effects of 0.5–2.0% v/v halothane on large K^{+} -induced catecholamine secretion and influx of $^{45}\text{Ca}^{2+}$ in bovine adrenal medulla cells cultured for 3 to 7 days, Yashima, Wada and Izumi [138] did not find inhibition of VOCC-mediated $^{45}\text{Ca}^{2+}$ uptake into these cells. As shown by the inhibitory effect of the L type Ca^{2+} channel blocker verapamil, this $^{45}\text{Ca}^{2+}$ uptake was largely L type VOCC-dependent. In freshly prepared suspensions of bovine adrenal medulla cells containing more than 60% chromaffin cells, however, halothane, enflurane, isoflurane and methoxyflurane produced significant dose-dependent suppression of $^{45}\text{Ca}^{2+}$ influx evoked by depolarizing concentrations of K^{+} [99]. The inhibition of the voltage-gated Ca^{2+} influx seen with all four agents was sufficient to account for the decrease in K^{+} -induced catecholamine secretion. The type of VOCC involved was not characterized [99], although the occurrence of L and N type channels has been described in bovine adrenal medulla cells [16]. Also in this study, the large K^{+} -stimulated $^{45}\text{Ca}^{2+}$ uptake was less sensitive to the inhibitory action of anaesthetics than that evoked by stimulation of the nicotinic acetylcholine receptor with carbachol. Thus in these cell preparations, the IC_{50} values for inhibition of large K^{+} -stimulated Ca^{2+} influx were above the clinical relevant concentration range of the anaesthetics tested [99].

In contrast, studies using well characterized clonal neurosecretory cell lines revealed a great sensitivity of LVA and HVA VOCC to clinical concentrations (i.e. $< 1 \text{ mmol litre}^{-1}$ aqueous concentration) of volatile anaesthetics [39, 57, 58, 119]. In the clonal rat pheochromocytoma cell line PC12, which possesses properties of adrenal chromaffin cells and sympathetic neurones, halothane, enflurane, isoflurane and methoxyflurane showed dose-dependent, reversible inhibition of large K^{+} -induced Ca^{2+} influx

through dihydropyridine sensitive L type VOCC [57, 58], that was paralleled by inhibition of catecholamine secretion [58]. Half-maximal inhibition occurred within the clinically relevant concentration range ($< 1 \text{ mmol litre}^{-1}$). The nicotinic receptor-mediated Ca^{2+} response was equally sensitive to the volatile anaesthetics [58]. Since at least two independent Ca^{2+} channels participated in the nicotinic increase in Ca^{2+} , one of which proved insensitive to nifedipine, the inhibitory action of the anaesthetics was not restricted to the L type HVA Ca^{2+} channel [58]. Studies in nerve growth factor differentiated PC12 cells, which also possess a large number of dihydropyridine insensitive VOCC [123], revealed dose-response curves indistinguishable from those obtained in undifferentiated cells, thus providing further evidence that the inhibitory effect of volatile anaesthetics is not restricted to L type VOCC [58, unpublished observations]. To date, however, no data have been reported on the sensitivity of N type or P type VOCC to volatile anaesthetics.

In the clonal pituitary cell line GH3, clinical concentrations of halothane ($0.5 \text{ mmol litre}^{-1}$) inhibited K^{+} ($10 \text{ mmol litre}^{-1}$)-induced increase in cytoplasmic Ca^{2+} and the corresponding prolactin secretion, without affecting resting Ca^{2+} concentrations, membrane potentials or the secretory apparatus itself [119]. These biochemical results have been confirmed by extensive electrophysiological experiments carried out in the same GH3 cell line, using standard whole cell clamp techniques to examine the effects of a wide concentration range of halothane ($0.1\text{--}5 \text{ mmol litre}^{-1}$) on LVA and HVA Ca^{2+} currents [39]. Halothane reduced both the LVA and HVA component of the Ca^{2+} current. The fact that both currents proved substantially more sensitive to halothane than either voltage-dependent Na^{+} or K^{+} currents in the same preparation [39] supports the view that volatile anaesthetics do not produce non-specific alteration in membrane ion conductance. Investigating possible mechanisms underlying the observed reduction of HVA Ca^{2+} currents, the authors concluded that halothane acts directly on that Ca^{2+} channel. In particular, it was possible to rule out Ca^{2+} -mediated inactivation of HVA current, the involvement of G proteins and rundown of the channel by dephosphorylation [39].

Interestingly, LVA (T type) Ca^{2+} channels proved less sensitive than HVA channels with half-maximal inhibition of peak current at $1.3 \text{ mmol litre}^{-1}$ and $0.8 \text{ mmol litre}^{-1}$ of halothane, respectively [39]. In contrast to HVA currents, the reduction of LVA currents was associated with changes in activation and inactivation kinetics with only negligible changes in the peak current amplitude, thus resembling the effects of volatile anaesthetics on Na^{+} current kinetics. The observed increases in activation and inactivation rates, however, were not sufficient to account for the reduction of peak LVA current. Therefore, reductions in the current flow through LVA channels or in the number of open LVA channels were suggested as additional inhibitory mechanisms [39].

In neonatal and adult rat dorsal root ganglion cells, halothane and isoflurane reduced the amplitude

of LVA Ca^{2+} currents more effectively than in GH3 cells, showing a half-blocking concentration of halothane of approximately $0.1 \text{ mmol litre}^{-1}$ for neonatal cells [124]. Since the activation kinetics of the two LVA currents were quite different [39, 124], the observed differences in halothane sensitivity suggest that LVA channels may be heterogeneous between cell types and possibly even within a single cell [1, 112].

In contrast to GH3, where the HVA Ca^{2+} current was found to be markedly more sensitive to halothane than the LVA component [39], the HVA Ca^{2+} current in rat sensory neurones was insensitive, showing only 8% reduction at $0.25 \text{ mmol litre}^{-1}$, 23% reduction at $0.5 \text{ mmol litre}^{-1}$ and half-maximal reduction at approximately $1.5 \text{ mmol litre}^{-1}$ of halothane [124]. Taking into account the different halothane sensitivities reported for HVA Ca^{2+} channels in cells of distinct origin (dog [120] and bovine adrenal medulla [99, 138], rat PC12 cells [57, 58], rat pituitary GH3 cells [39, 119], rat sensory neurones [124]), these findings also suggest selective action of halothane on distinct members of a heterogeneous family of HVA Ca^{2+} channels. Whether or not channel proteins with distinct structural differences are responsible, or whether or not non-channel proteins or modulatory mechanisms, such as protein kinases, second messengers or G proteins may underlie these different sensitivities to the inhibitory effect of volatile anaesthetics remains unclear. The difference in sensitivity to halothane found between HVA channels of GH3 *vs* dorsal root ganglion cells, for example, may be a result of structural or functional differences of the participating Ca^{2+} channels (N and L type VOCC?).

Nevertheless, there is growing evidence from experiments with isolated neural cells and cell lines of neural crest origin, that at least some VOCC are very sensitive to the inhibitory effects of volatile anaesthetics. This suppression of Ca^{2+} currents may not only be crucial in presynaptic nerve terminals, but inhibition of postsynaptic Ca^{2+} currents may also be of critical importance, for example by interfering with the formation of memory traces [72, 126]. It will be interesting to elaborate the functional or structural differences between anaesthetic sensitive and insensitive Ca^{2+} channels at a molecular level. The approach will provide new insights into the structural prerequisites for anaesthetic susceptibility, and may also reveal differences in channel regulation [40] and modification by second messenger mechanisms [113]. Thus different VOCC possessing different susceptibilities to anaesthetic inhibition may exist in a single cell, as well as in different cell types and species [26].

LIGAND OPERATED CALCIUM CHANNELS

The binding of certain neurotransmitters to specific receptors also activates cation channels that accommodate Ca^{2+} as well as many other ions [78]. These ligand operated Ca^{2+} channels represent another pathway for Ca^{2+} entry that may be important in both presynaptic and postsynaptic regulation of second messenger Ca^{2+} . Activation of

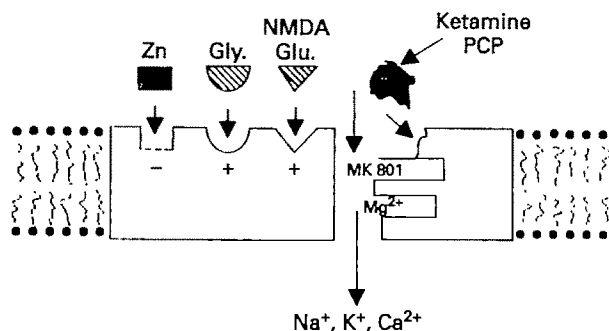


FIG. 3. Schematic representation of the N-methyl-D-aspartate (NMDA) receptor with its modulatory sites. The receptor contains two binding sites for agonists such as glutamate (Glu.) and NMDA. These sites are modulated positively by glycine (Gly.) and negatively by Zn^{2+} . The dissociative anaesthetics ketamine and phencyclidine (PCP) probably bind to a site close to the entrance of the cation channel, which is permeable to Na^+ , K^+ and Ca^{2+} . Binding of the anaesthetics blocks channel function. The Mg^{2+} binding site is probably located deeper in the cation channel. The presence of Mg^{2+} strongly inhibits channel activation. The binding site for the non-competitive NMDA antagonist MK 801 is also located in the channel pore.

receptors that are part of, or closely linked to, their respective ionophores, such as the nicotinic acetylcholine receptor or the receptor for the excitatory amino acid N-methyl-D-aspartate (NMDA), leads directly to the opening of the channel that is more or less selective for Ca^{2+} entry into the cytoplasm. Simultaneously VOCC are activated also [35, 88].

Finally, channels activated from the cytoplasmic side, probably by second messengers, may also participate in transporting Ca^{2+} into the neurone. For certain G protein-coupled receptors [14], previously called second messenger operated Ca^{2+} channels that are insensitive to both dihydropyridines and ω -CgTx, have been described [78]. The exact mechanisms and the regulation of these channels are not clear. Unfortunately, the nomenclature concerning ligand activated Ca^{2+} channels is not uniformly used in current literature. Both NMDA and nicotinic acetylcholine receptor activated channels and so-called second messenger operated Ca^{2+} channels are often referred to as receptor operated Ca^{2+} channels (ROCC). In this review, the term receptor operated Ca^{2+} channel is applied exclusively to those formerly called second messenger operated channels (muscarinic receptor, endothelin 1 receptor), whereas ionotropic receptors (e.g. NMDA, nicotinic acetylcholine) are designated as ligand activated channels (LAC). As demonstrated in pituitary GH3 cells, thyrotropin-releasing-hormone-activated accumulation of IP3 and intracellular Ca^{2+} release may also be associated with sustained Ca^{2+} influx through HVA VOCC, suggesting interrelations between Ca^{2+} entry pathways activated by agonists and by depolarization [35, 119].

Nicotinic receptor-induced Ca^{2+} influx

The depressant effect of volatile anaesthetics on nicotinic acetylcholine receptor-induced catecholamine secretion was a consistent finding in medulla cells and neurosecretory cell lines from different

species [33, 34, 58, 99, 120, 138]. The nicotinic receptor itself, the receptor operated cation channel, membrane depolarization and the L type VOCC have all been discussed as potential target sites for volatile anaesthetics.

Early work on cholinergic receptor stimulated catecholamine secretion from perfused dog adrenal medulla revealed indirect evidence that the halothane susceptible process might be Ca^{2+} influx through receptor linked Ca^{2+} channels [120]. However, in this study, no Ca^{2+} measurements were performed. In cultured bovine adrenal medulla cells, 0.5–2% v/v halothane inhibited the nicotinic receptor-mediated influx of $^{45}Ca^{2+}$ in a dose-dependent, reversible and non-competitive manner. This Ca^{2+} influx proved sensitive to the inhibitory actions of the organic Ca^{2+} channel blockers diltiazem, verapamil and nifedipine, and was thus mainly mediated via L type VOCC [138].

Similarly, Pocock and Richards [99] observed a marked depression of nicotinic acetylcholine receptor stimulated $^{45}Ca^{2+}$ influx and catecholamine secretion in freshly isolated bovine medulla cells at clinically relevant concentrations of halothane, enflurane, isoflurane and methoxyflurane. Since the $^{22}Na^+$ influx through the nicotinic LAC was also blocked by the anaesthetics, this inhibition was sufficient to account for the marked decrease in Ca^{2+} influx and catecholamine secretion. Based on the lack of comparably strong inhibition of K^+ activated VOCC in these cells, interaction of volatile anaesthetics with either the nicotinic receptor, the processes associated with its activation, or with the coupling between receptor and Ca^{2+} channels, has been assumed [99].

In the rat pheochromocytoma cell line PC12, the nicotinic acetylcholine receptor stimulated Ca^{2+} influx was only partially sensitive to nifedipine and involved at least two distinct pathways for Ca^{2+} entry: L type VOCC and the nicotinic LAC, which proved insensitive to nifedipine and has been shown to mediate nicotinic acetylcholine receptor-evoked secretion, even in the absence of external Na^+ [108, 134]. The fact that the nicotinic acetylcholine receptor-induced Ca^{2+} increase was at least as sensitive to clinical concentrations of halothane and isoflurane as the VOCC-mediated Ca^{2+} response, also supports the assumption that the nicotinic LAC, the functionally linked dihydropyridine resistant VOCC, or both, or the nicotinic acetylcholine receptors are sensitive targets for volatile anaesthetics [58].

NMDA activated channels

L-glutamate, or a similar molecule, is probably the most widely distributed excitatory neurotransmitter in the brain. This amino acid can activate a variety of receptor subtypes, including NMDA, kainate, quisqualate and AMPA receptors [70, 76, 117]. In this review, we focus exclusively on the NMDA receptor because it has been studied extensively and activation results in large Ca^{2+} transients inside the cell.

The NMDA receptor ionophore consists of a non-specific cation channel permeable to Na^+ , K^+ and Ca^{2+} . The NMDA receptor-ionophore complex shows pharmacological properties completely differ-

ent from VOCC. Although the LAC is blocked in a voltage-dependent fashion by small concentrations of Mg^{2+} , gating of the NMDA channel is not a voltage-dependent process. There are two agonist binding sites for which L-glutamate is presently the highest affinity endogenous ligand. Furthermore, it has a binding site for the co-agonist glycine [128], and sub-micromolar concentrations of glycine enhance greatly receptor activation. The dissociative anaesthetic ketamine and the psychotropic drug phencyclidine antagonize NMDA-induced depolarizations on rat spinal cord neurones non-competitively [2]. Their binding sites may be located near or in the NMDA receptor channel. A schematic presentation of the NMDA receptor and its binding sites is shown in figure 3. The glutamate-induced Ca^{2+} increase depends on the presence of Ca^{2+} in the extracellular space and is a result of both the activation of LAC and VOCC [88]. The Ca^{2+} increase inside the cell may in turn synergize with activated protein kinase [76]. NMDA receptor ionophores may be involved in excitatory synaptic transmission and in long-term potentiation [46], which is a sustained increase in synaptic efficacy after brief tetanic stimulation of certain excitatory pathways and has been assumed to be involved in information storage and memory [126].

For ketamine, there is good evidence that one site of action is the NMDA receptor-ionophore complex [137]. The intoxicating effect of several alcohols corresponds with their inhibition of the NMDA activated current [67]. The actions of volatile anaesthetics on the NMDA activated channel are not well documented and conflicting observations have been made. Whereas there is indirect evidence that volatile anaesthetics depress glutamate [107] or NMDA mediated neurotransmission [23, 71, 72, 102, 111], other reports [10, 96] do not find it influenced. These discrepancies may relate to differences between species, preparations or experimental techniques. Interestingly, enflurane inhibited glutamate stimulated binding of the non-competitive NMDA antagonist MK-801 in rat cerebral cortex, and this inhibition could be antagonized by glycine [73]. Since, however, unspecific actions not located at the NMDA receptor-channel cannot be excluded, direct measurements of the effects of volatile anaesthetics on NMDA-evoked ion currents are necessary.

In the neocortex, the actions of glutamate or NMDA were attenuated sharply by isoflurane in a concentration-dependent manner [101]. Isoflurane and halothane suppressed the glutamate stimulated intracellular Ca^{2+} increase mediated by quisqualate or NMDA receptors in cultured hippocampal neurones, and NMDA or large K^{+} -induced Ca^{2+} responses were similarly depressed in these cells [102, 103]. Thus the reduced Ca^{2+} influx through both NMDA receptor operated channels and VOCC in the postsynaptic membrane may at least partly contribute to the observed depression of glutamate sensitivity by volatile anaesthetics. However, direct measurements of the inhibitory effects of volatile anaesthetics on NMDA activated currents have not yet been performed.

RECEPTOR OPERATED CALCIUM CHANNELS

In addition to intracellular Ca^{2+} release, agonist receptors linked to inositol phospholipid hydrolysis also cause the opening of cation channels permeable to Ca^{2+} [79]. These so-called receptor operated Ca^{2+} channels (ROCC) have been postulated to occur in most cells [90]. For example, the activation of ROCC has been observed with histamine [12], bradykinin [31], muscarine [51], thrombin [141], endothelin [20], ATP [52], ADP [110] and platelet derived growth factor [122]. More agonists and neurotransmitters linked to ROCC will probably be found in the near future.

ROCC are not inhibited by dihydropyridines or ω -CgTx (table II), which are inhibitors of L type and N type VOCC [87]. Ion flux through ROCC is, however, sensitive to Ni^{2+} [50] or La^{3+} [87]. Some, but not all, are sensitive to the imidazole derivative SK&F 96365 [80] and SC 38249 [22], indicating that different subtypes of ROCC may exist. Most neurotransmitters that activate ROCC produce a biphasic Ca^{2+} response when added to their target cells [20, 52, 110, 122]. The first part of the response consists of rapid production of IP_3 which releases Ca^{2+} from the endoplasmic reticulum, followed by slower sustained influx of Ca^{2+} from the extracellular space. Inhibition of receptor operated Ca^{2+} influx generally prevents the physiological response, indicating that Ca^{2+} influx is an essential part of the receptor response and not merely replenishment of Ca^{2+} stores.

There is considerable uncertainty about the mode of ROCC activation. Initially, activation by an increase in cytoplasmic Ca^{2+} , IP_3 or its phosphorylation product inositol 1,3,4,5 tetrakisphosphate was suggested. However, more recent data suggest that ROCC may be directly coupled to the receptors via G proteins (fig. 4) [17, 78]. Unfortunately, it is difficult to study these channels electrophysiologically, but ROCC activity may be monitored indirectly using cells loaded with the Ca^{2+} indicator fura-2. Stimulation of receptors coupled to ROCC in such cells in a Ca^{2+} free medium uncovers Ca^{2+} release from intracellular stores. After addition of Ca^{2+} to the extracellular medium, Ca^{2+} flows through ROCC, thereby increasing fura-2 fluorescence. Using this experimental protocol it has recently been shown that clinical concentrations of volatile anaesthetics depress the Ca^{2+} increase mediated by ROCC [unpublished observation]. Halothane and isoflurane inhibited the ROCC-mediated Ca^{2+} increase in a dose-dependent manner at clinical concentrations (IC_{50} approximately 2 MAC) [unpublished observations]. The investigation of the

TABLE II. *Pharmacological properties of receptor operated calcium channels*

Binding of a receptor agonist prerequisite for activation
Depolarization not required for activation
Insensitive to dihydropyridine Ca^{2+} channel blockers
Insensitive to omega-conotoxin
Sensitive to the imidazole derivative SK&F 96365

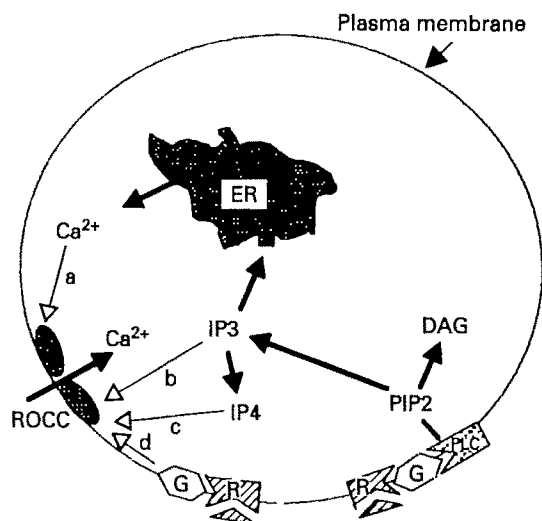


FIG. 4. Possible pathways for activation of receptor operated calcium channels (ROCC). It has been suggested that ROCC are activated by an increase in $[\text{Ca}^{2+}]$, (a), by inositol 1,4,5 triphosphate (IP_3) (b) or its phosphorylation product inositol 1,3,4,5 tetrakisphosphate (IP_4) (c). More recent data suggest a role for G proteins in channel activation (d).

exact inhibitory mechanism was complicated by the fact that volatile anaesthetics increased the cytoplasmic Ca^{2+} concentration in glioma cells, thereby probably exerting a negative feedback on Ca^{2+} influx through ROCC.

RECEPTOR STIMULATED INTRACELLULAR CALCIUM RELEASE

Binding of hormones and neurotransmitters to G protein-coupled membrane receptors activates phosphoinositide hydrolysis. Breakdown of PIP_2 generates IP_3 (which mobilizes Ca^{2+} from intracellular stores [11]) and diacylglycerol (DAG), which activates protein kinase C by increasing the affinity of the enzyme for Ca^{2+} and phospholipid [92, 93]. The mechanism of intracellular release has been investigated in detail, and the IP_3 receptor purified, cloned and its intracellular distribution established in cerebellar Purkinje neurones. Phosphoinositide hydrolysis is one major mechanism for transmembrane signal transduction in neural cells, and muscarinic acetylcholine receptors, for example, are coupled to PIP_2 hydrolysis in brain and in several neural cell lines (e.g. PC12) [14, 47, 132].

Differential effects of volatile anaesthetics on muscarinic and nicotinic acetylcholine receptor-mediated stimulus-secretion coupling have been reported in perfused dog adrenal medulla [120]. As mentioned above, nicotinic acetylcholine receptor-induced catecholamine release proved very sensitive to clinical concentrations of halothane, whereas muscarine-induced secretion was significantly more resistant.

Since neither Ca^{2+} influx nor intracellular Ca^{2+} increase could be measured in that study [120], the possible involvement of Ca^{2+} remained unclear, until Kress and co-workers [57, 58] showed almost complete resistance to volatile anaesthetics of internal Ca^{2+} increase and consecutive catecholamine se-

cretion in response to muscarinic receptor stimulation in PC12 cells. Unlike bovine medulla cells, PC12 cells express both functional muscarinic and neuronal nicotinic acetylcholine receptors, and both are capable of stimulating secretion of catecholamines [58]. Neither halothane, enflurane nor isoflurane were able to depress significantly the IP_3 -mediated intracellular Ca^{2+} increase within a clinical concentration range (0.5–5% v/v). Muscarinic receptor-induced secretion was even enhanced in a dose-dependent manner in the presence of anaesthetics [58], perhaps reflecting the parallel increase in resting cytoplasmic Ca^{2+} concentrations observed at larger concentrations of volatile anaesthetics (see above) [125].

Comparable investigations on IP_3 -mediated intracellular Ca^{2+} release in the clonal rat pituitary cell line GH3 confirmed its relative resistance to halothane [119]. In these cells, the TRH-induced signal cascade is initiated by activation of phospholipase C via a G protein-linked process resulting in IP_3 -mediated Ca^{2+} release from intracellular stores. Halothane had little effect on the initial Ca^{2+} peak and the resulting early phase of secretion, consistent with the observation that halothane did not alter TRH stimulated phosphoinositide hydrolysis in these cells [119]. These data clearly indicated that the function of receptors and G proteins coupled to the receptors, phospholipase C and IP_3 -induced Ca^{2+} release were well preserved in the presence of clinical concentrations of halothane.

To date, very few other reports exist on the effect of volatile anaesthetics on phospholipid-inositol hydrolysis. Halothane did not affect inositol phosphate accumulation in slices of rat brain after stimulation with noradrenaline, carbachol, histamine or 5-hydroxytryptamine [8]. Halothane and alcohols stimulated phospholipase C in hepatocytes leading to increased concentrations of IP_3 and intracellular Ca^{2+} [44]. Stimulation of phospholipase C most probably occurred via activation of an as yet uncharacterized G protein (not G_i or G_o) in these cells.

In contrast, clinical concentrations of halothane inhibited inositol phosphate formation and cytoplasmic Ca^{2+} signalling induced by arginine vasopressin in a cultured vascular smooth muscle cell line [116]. In these cells, Ca^{2+} release, rather than Ca^{2+} influx, was more sensitive to the inhibitory effect of halothane. The altered Ca^{2+} homeostasis in the presence of the anaesthetic was explained by depletion of Ca^{2+} from intracellular stores, as well as by inhibition of inositol phosphate formation. However, the effects of halothane were not specific to the G protein-linked pathway, but also included responses that are thought to be void of G protein involvement. Thus either phospholipase C itself, PIP_2 as a substrate of phospholipase C, or metabolism of inositol phosphates may be considered possible targets for halothane in vascular smooth muscle.

Although in mouse myocardium a possible interaction of halothane with G proteins was shown [133], in neural cells there is to date no convincing evidence that clinical concentrations of volatile anaesthetics interfere directly with G protein-

coupled processes involved in intracellular Ca^{2+} homeostasis [77]. For rat brain membranes it was shown that halothane disrupted the interaction of serotonin 1A and adenosine A1 receptors with G proteins [74]. In neural membranes from rat brain stem, volatile anaesthetics were reported to abolish guanine nucleotide regulation of antagonist, but not agonist binding to muscarinic receptors [3, 4]. Since agonist binding was largely unaffected, the physiological relevance of these phenomena remains doubtful.

CONCLUSIONS

Focusing particularly on neurones and neurosecretory cells, the effects of volatile anaesthetics on transmembrane Ca^{2+} signalling were reviewed. Cytoplasmic Ca^{2+} increases when it is released from intracellular stores or when calcium ions flow through various types of voltage operated or ligand activated channels, and each of these processes may be influenced by volatile anaesthetics.

Intracellular Ca^{2+} homeostasis is affected even in resting cells; volatile anaesthetics such as halothane increase the resting cytoplasmic Ca^{2+} concentration, either by augmentation of the intracellular release or by enhanced influx from outside the cell. Depending on the Ca^{2+} activated effector mechanisms involved, this Ca^{2+} increase inhibits or activates different enzymes or channel proteins. In this respect, very little is known about anaesthetic effects on Ca^{2+} ATPase or Na^+ - Ca^{2+} exchange in neural cells.

There is good evidence that volatile anaesthetics inhibit T type and L type voltage operated Ca^{2+} channels, not only in myocytes [15, 29], but also in neurosecretory cells and neurones. However, because of our limited understanding of the structure and function of the different Ca^{2+} channel subtypes, especially within the CNS, controversial results on the interactions between volatile anaesthetics and VOCC have been reported with different cell types even though the channels were indistinguishable by pharmacological and electrophysiological means. In the near future, molecular biology will help to solve such discrepancies, which may be caused by minor differences in the genetically determined molecular structure of channel proteins or the presence of modulating subunits in various tissues. The different halothane sensitivities observed for VOCC in adrenal medulla, PC12, GH3 and dorsal root ganglion cells suggest selective action of volatile anaesthetics on distinct members of a heterogeneous family of VOCC. To date, nothing is known about actions of volatile anaesthetics on N type or P type VOCC. Within the group of ligand activated channels, only Ca^{2+} influx linked to the nicotinic receptor was shown to be uniformly depressed by the volatile substances tested so far, whereas there is some uncertainty as to the effects of NMDA receptor linked channels or so-called receptor operated channels.

In neural cells, inositol phosphate-mediated intracellular Ca^{2+} release appeared to be resistant to inhibition by volatile anaesthetics, whereas in cultured vascular smooth muscle cells it was not.

Whether or not this is because of different sensitivities of G proteins or G protein-coupled processes remains unclear.

In conclusion, volatile anaesthetics have been shown to affect distinct voltage operated and ligand activated Ca^{2+} channels in neural and non-muscular cells, suggesting modulating or inhibitory effects on signal transduction and neuronal function on both presynaptic and postsynaptic levels. Rapid advances in molecular biology will contribute considerably to our understanding of structure, function and regulation of these Ca^{2+} channels and help to elucidate the molecular basis underlying their interactions with volatile anaesthetics.

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EFFECTS OF GENERAL ANAESTHETICS ON LIGAND-GATED ION CHANNELS

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The site at which anaesthetics act within the central nervous system (CNS) has been the subject of research for almost 100 years. Most success has been obtained in defining the physical nature of the site [70]. For example, the well established correlation of anaesthetic potency to fat solubility indicates that the site of action is hydrophobic. Research using anaesthetics with unusual solubility properties (sulphur hexafluoride and carbon tetrafluoride) failed to provide support for the alternative hypothesis that interaction within the aqueous phase of the CNS was responsible for anaesthesia [45, 46]. It is now generally agreed that interaction at a hydrophobic site is involved, but debate continues as to whether or not this is within the membrane lipids or at a hydrophobic region within specific proteins. Evidence for the latter view has been provided by the fact that bacterial and firefly luciferases are sensitive to anaesthetics with potency ratios comparable to those of mammalian anaesthesia [21, 22, 32, 42, 43]. However, there is no evidence for a substance related to the luciferases within the mammalian CNS and for the protein model of general anaesthesia to be advanced such a target site must be identified.

General anaesthetics may affect signal transmission by altering action potential propagation or synaptic transmission or, indeed, both. At clinically relevant concentrations, general anaesthetics appear primarily to affect synaptic processes [5, 53, 58–61], although impulse conduction in small unmyelinated fibres is reduced [4]. There is some evidence that anaesthetics may inhibit neurotransmitter release also [56], which if it were to occur at an excitatory synapse could produce the necessary depression of synaptic transmission.

Anaesthetics also have effects on the postsynaptic neurotransmitter receptor/ion channel complex. Much of the early work concentrated on the nicotinic acetylcholine receptor as a model for ligand-gated ion channels in general. However, attention is now being devoted to the receptors activated by excitatory amino acids, which probably form the majority of the fast excitatory synapses in the CNS, and to the principal inhibitory receptors, activated by γ -aminobutyric acid (GABA) and glycine.

ACETYLCHOLINE-ACTIVATED ION CHANNELS

The effects of anaesthetics on the acetylcholine receptor have been studied extensively [13, 18, 23, 24, 44, 48]. The reason for the widespread use of this receptor is that the electric tissue of *Torpedo* provides a source of receptor-rich membranes and, furthermore, the receptor derived from this tissue is homologous with that at the mammalian neuromuscular junction [2] and with that from the human brain [74].

The large density of acetylcholine receptors in the electric tissue has made it possible to use ion flux techniques to investigate the effect of anaesthetics on the acetylcholine receptor [44, 48]. Rapid kinetic studies, in which the flux of a radioactive cation (typically $^{86}\text{Rb}^+$) is measured, have been performed and the results, in the presence and absence of anaesthetics, have been interpreted on the basis of a model of the acetylcholine receptor in which there are two resting states for the receptor, normal and desensitized, and that when two molecules of acetylcholine bind there is rapid transition to an open channel state. Normally, acetylcholinesterase rapidly removes acetylcholine and the channel returns to the resting state. However, there is the possibility that an anaesthetic molecule could block the open channel, preventing return to the resting state. It is assumed that the channel must be unblocked before it can return to the resting state (sequential block model) [48]. All general anaesthetics have been shown to increase the proportion of receptors in the desensitized state. Furthermore, it appears that this action may be mediated by some non-specific membrane perturbation [48]. In addition, most general anaesthetics block the ion channel also, although it is not clear by what mechanism [23]. However, it has been established recently that the site at which general anaesthetics block the channel is different from that at which local anaesthetics bind to block the channel [48].

Electrophysiological measurements of single channel activity, under conditions of patch-clamp, have shown that general anaesthetics cause the channel to flicker, that is openings become more frequent, briefer and appear to be grouped into bursts [18, 24, 50]. If a simple, sequential channel block model of anaesthetic action is assumed, in which the

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KEY WORDS

Anaesthetic action. Electrophysiology: ligand-gated channels. General anaesthesia: enflurane, halothane, isoflurane, propofol.

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anaesthetic may only bind to the open channel and the flickering is caused by the open channel being repeatedly blocked, it follows that the mean burst duration should increase linearly with increasing concentration of anaesthetic. Experimental evidence indicates that this does not occur, rather the bursts get shorter with increasing concentration [18]. To account for this it has been proposed that the open blocked state can be terminated by the closing of the blocked channel. This extension to the simple sequential block model of the mode of action does predict correctly the concentration-dependent behaviour of the burst duration and the number of openings/burst [18, 50].

However, even this extended model for the interaction of anaesthetics and the nicotinic acetylcholine receptor cannot be complete. For example, the frequency of bursts increases in the presence of anaesthetics [18]. It is not clear at present if this occurs as a result of changes in acetylcholine binding or as a result of changes in the rate of induced conformational change. Finally, anaesthetics accelerate the fast desensitization process that occurs in the presence of high concentrations of acetylcholine [18]. This implies that the model will have to incorporate modified desensitization rates or, alternatively, additional desensitized states.

GLUTAMATE-ACTIVATED ION CHANNELS

Glutamate-activated ion channels are obvious targets for general anaesthetics because they are probably the principal neurotransmitter receptors that mediate synaptic excitation in the vertebrate CNS. Glutamate receptors have been categorized, on the basis of their preferred agonist, into a group of three ionotropic (i.e. gated by the neurotransmitter) receptors (NMDA (N-methyl-D-aspartate), KA (kainate) and AMPA (quisqualate)) and two metabotropic (i.e. accessing the metabolic machinery of the cell) G protein coupled subtypes (L-AP4 (2-amino-4-phosphonobutyrate) linked to the hydrolysis of cGMP and ACPD (trans-1-amino-cyclopentane-1,3-dicarboxylate) linked to inositol phosphorate diacylglycerol formation).

The three ionotropic receptors operate on a fast (ms) time scale, whilst the two G protein coupled receptors operate on a time scale of hundreds of milliseconds to seconds [26]. NMDA receptors show a voltage-dependent Ca^{2+} permeability with a relatively slow rise time and a decay time of several hundred milliseconds [20, 63]. AMPA receptors conduct mainly Na^+ currents [14]. The distinction between AMPA and KA receptors is at present unclear. It was made on the basis of discrete high affinity binding sites for these two ligands [15]. However, high affinity binding sites need not necessarily imply a functional receptor/channel complex.

Recent DNA cloning experiments have revealed a large number of receptor subtypes [26, 73]. At present there are approximately 10 ionotropic receptor subunits known, although not any subunit may assemble with any other to form a functional glutamate-activated channel with distinct properties.

It appears that there may be four distinct AMPA receptors, three KA receptors and one NMDA receptor, together with three high affinity KA subunits which do not form a functional receptor. Further experimental progress is required before the complete categorization of this receptor family may be achieved.

In mice, the non-competitive NMDA antagonists MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo-(a,d)cyclo-hepten-5,10-imine), phencyclidine and ketamine increase the potency of the general anaesthetics ethanol, pentobarbitone, halothane and diethyl ether, in parallel with their potency as NMDA antagonists [16].

Ethanol 30 mmol litre⁻¹, a concentration which produces intoxication in man, induces 50% inhibition in NMDA-induced currents in cultured hippocampal neurones but less than 10% inhibition in AMPA- or KA-induced currents [77]. The IC_{50} values for currents elicited by NMDA for a range of alcohols (methanol, ethanol, 1-butanol, isopentanol) correlated with both the membrane/buffer partition coefficient and the ED_{50} for intoxication for these alcohols [77]. Similarly, the volatile anaesthetic diethyl ether, inhibits currents elicited by NMDA but not AMPA or KA [77].

In contrast, pentobarbitone and phenobarbitone do not affect currents elicited by NMDA. However, pentobarbitone inhibits KA and AMPA currents elicited in cultured hippocampal cells (IC_{50} approximately 50 $\mu\text{mol litre}^{-1}$) [77]. The inhibition was antagonized rapidly and not voltage dependent, suggesting that the block does not occur deep within the channel [77]. Phenobarbitone inhibits similarly KA and AMPA currents elicited in cultured hippocampal cells [77]. The current elicited by KA in *Xenopus* oocytes, in which glutamate receptors are induced by microinjection with mRNA from rat brain, is also inhibited by phenobarbitone [17]. The inhibition by phenobarbitone of currents elicited in oocytes was not sensitive to pressures up to 135 bar [17].

The steroid anaesthetic alphaxalone had no effect on NMDA-, AMPA- or KA-induced currents [77].

Experiments using cultured hippocampal cells have shown that isoflurane, at concentrations greater than 2 mmol litre⁻¹, induces channel flickering in NMDA receptors, with a decrease in channel open time that is dependent on the dose of anaesthetic [79]. Burst durations also showed a dose-dependent decrease with no decrease in apparent single channel conductance. These actions of isoflurane are different to its actions on the acetylcholine receptor. For the acetylcholine receptor channel, small concentrations of isoflurane produced a dose-dependent decrease in channel open times and the frequency of channel openings increased [12].

In another study using cultured rat hippocampal CA1 neurones, the intermediate chain length n-alkanols decreased both the mean channel open time and the probability of opening of single NMDA channels [41]. There was no significant effect on the amplitude of single channel currents or channel conductance. No evidence for channel block (flickering) was observed.

GABA-ACTIVATED ION CHANNELS (see also Goodchild [27])

GABA receptors are distributed widely throughout the CNS and are responsible for both pre- and postsynaptic inhibition. GABA receptors have traditionally been divided into ionotropic GABA_A and metabotropic GABA_B subtypes. GABA_A receptors are activated by muscimol, inactivated by bicuculline and sensitive to benzodiazepines, whereas GABA_B receptors are activated by baclofen, inactivated by phaclofen and unaffected by benzodiazepines. GABA_A receptors conduct Cl⁻ ions [1, 6, 62] whereas the GABA_B receptor is coupled to Ca²⁺ channels, K⁺ channels and G proteins [9, 19, 34, 51, 78].

Recently, the GABA_A receptor has been separated into a number of subtypes, based on the multiple α, β, γ subunit sequences of its cloned cDNA [49, 57, 66–68]. Attempts to establish similar subtype separation for the GABA_B receptor have not been successful [8, 76].

One of the key features of GABA_A receptors is the potentiation observed in the presence of barbiturates. Inhibitory postsynaptic currents or potentials recorded from various regions of the brain are potentiated [24, 25, 64, 65], as is the GABA activated response from cultured neurones [38, 53]. Barbiturates affect allosterically the binding of GABA to the receptor site, decreasing the kinetics and increasing the affinity [52]. Single cell patch-clamp recording from cultured mouse spinal cord neurones showed that barbiturates increased the average channel open time and increased the mean number of openings per burst [40]. Both these contributions are important for the observed potentiation of the response to GABA.

Steroid anaesthetics similarly potentiate the function of GABA_A receptors and affect allosterically the binding of GABA. However, it has been established that the steroid anaesthetics bind to a different domain on the protein when compared to barbiturates [52]. Steroid anaesthetics, like barbiturates, prolong burst duration, as observed by single channel recording under patch-clamp conditions [33].

The volatile general anaesthetics halothane, enflurane and isoflurane, at concentrations equivalent to 2 MAC, have three effects on the response of GABA_A receptors. They potentiate the sustained response in the presence of small concentrations of GABA, accelerate the decay phase and suppress the steady state current activated by large concentrations of GABA [80]. With small concentrations of GABA, halothane did not alter the main conductance of a single channel but did lengthen the burst duration, shorten the interburst interval and prolong the open time [80]. To explain these actions it has been suggested that the volatile anaesthetics alter the gating properties of the receptor complex and increase the affinity of GABA for the receptor [80]. This is also consistent with potentiation of the peak current elicited by large concentrations of GABA but not, however, with suppression of the steady state current. Whether or not the effect on the steady state current is a result of enhancement of desensi-

tization processes or to differential effects on different receptor subtypes [80] awaits clarification.

GLYCINE-ACTIVATED ION CHANNELS

Glycine is the postsynaptic inhibitory neurotransmitter in the brain stem and spinal cord. The chloride channel activated by glycine has similar conductance properties to that activated by GABA [6, 7]. The glycine receptor has been isolated from mammalian CNS tissue and shown to comprise two integral membrane proteins (α and β) and a peripheral membrane protein [3, 28, 55]. It has been suggested that the channel core is a pentameric arrangement of three α and two β subunits [37]. This subunit arrangement closely resembles that of the nicotinic acetylcholine receptor [75]. As with other ligand-gated receptors, recent evidence from DNA sequencing experiments have indicated considerable heterogeneity in the glycine receptor subunits. To date, four variants of the α subunit have been identified but no variants of the β subunit have been identified [29, 35, 36].

In contrast to acetylcholine, glutamate and GABA receptors, relatively little work has been performed on the effect of anaesthetics on glycine receptors. It has been shown, using whole cell patch recording from cultured embryonic spinal neurones, that glycine-evoked currents were potentiated by propofol and chlormethiazole [30, 31], but not by pentobarbitone [30, 31] or alphaxalone [33]. Preliminary experiments recording whole cell currents from voltage clamped oocytes microinjected with mRNA derived from rat spinal cord have indicated that the strychnine-sensitive glycine-evoked current is inhibited by pentobarbitone [unpublished observations].

CONCLUSIONS

It is becoming increasingly apparent that the nicotinic acetylcholine, glutamate, GABA_A and glycine receptors are members of a ligand-gated receptor super family that evolved from a common ancestor [35, 66]. The transmembrane segments in particular appear to show significant amino acid sequence homology and topology, of four hydrophobic α helices. However, it is also clear that anaesthetics affect members of this receptor super family in different ways and that to use the nicotinic acetylcholine receptor as a model for which the interaction of anaesthetics with ligand-gated receptors in general can be elucidated is quite wrong.

Anaesthetics appear to act at the nicotinic acetylcholine receptor to block the channel, whereas not all anaesthetics block glutamate channels and the action at the GABA_A receptor appears to be an allosteric potentiation of GABA binding and, for some anaesthetics, an effect on the gating properties.

That large pressures (of the order of 10 MPa) will antagonize general anaesthesia [38] led to a theory of general anaesthesia that assumed that anaesthetics and pressure acted at a common site and via a common mechanism [47]. More recently it was observed that species that do not use glycine as a neurotransmitter do not exhibit pressure reversal of

anaesthesia [72] and that pressure, far from acting in a non-specific manner, appears to act in a highly selective way [10, 11, 71]. At the level of the neurotransmitter receptor-ion channel complex, it has been shown that the kainate channel is unaffected by pressure but that the glycine channel is highly pressure sensitive [16, 69]. However, this does not allow postulation that pressure and anaesthetics act via the same mechanism and the observed interaction could arise through a balance between inhibition and excitation in the CNS.

Given that the mechanisms that induce anaesthesia are not understood, it is difficult to establish if the effects observed in *in vitro* systems are related to general anaesthesia in animals. One important requirement is that a wide range of molecular structures should produce similar effects with potency ratios comparable to those observed *in vivo*. For glutamate, GABA_A and glycine receptors, such behaviour is not observed.

The simple anaesthetics (alcohols and volatile agents) affect NMDA receptors, whereas barbiturates affect AMPA and KA receptors and steroid anaesthetics do not appear to have any effect on glutamate receptors. Barbiturates have a discrete binding site at GABA_A receptors and affect allosterically GABA binding. Steroid anaesthetics appear to act similarly but utilize a different binding site. Volatile anaesthetics not only affect GABA binding but also gating properties. At the glycine receptor, some anaesthetics appear to potentiate glycine binding whilst others are either ineffective or even act to inhibit, either by channel blocking or by enhancing desensitization. Furthermore, it is possible that when it becomes feasible to experiment with specific receptor subtypes, differences in the action of anaesthetic on the different subtypes may be observed.

It appears that anaesthetics act differently on ligand-gated ion channels and that therefore the interaction between general anaesthetics and the ligand-gated ion channels studied so far cannot provide a basis for a general mechanism of anaesthesia. However, if we assume that different anaesthetics, or classes of anaesthetics, act via different mechanisms, the difficulty arises of identifying which of the different effects are responsible for general anaesthesia. Thus barbiturates block nicotinic acetylcholine channels, potentiate GABA binding, block KA and AMPA receptors and inhibit glycine receptors. All of these effects, apart from the effect on the glycine receptor, could be responsible for the induced loss of consciousness.

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SELECTIVE ACTIONS OF VOLATILE GENERAL ANAESTHETICS AT MOLECULAR AND CELLULAR LEVELS

N. P. FRANKS AND W. R. LIEB

General anaesthetic agents are non-specific in the sense that they have diverse shapes and sizes, and possess a variety of chemical groups. This structural and chemical non-specificity is often taken to imply an underlying non-selectivity in the action of these agents upon their targets, especially the primary molecular targets involved in the induction of general anaesthesia. However, recent work at the cellular and molecular levels has shown that, at clinical concentrations, these drugs can be surprisingly selective in their actions, substantially affecting some neurones but not others and some ion channels but not others. In addition, even when acting on a sensitive target, different general anaesthetics (including optical isomers of the same agent) can be unexpectedly selective in their actions.

This review focusses on volatile general anaesthetics, which (together with anaesthetic gases) are considered generally to be the least selective of all agents. In particular, we will consider the effects of the most popular currently used agents (halothane, isoflurane and enflurane), as well as anaesthetics such as diethyl ether, methoxyflurane and chloroform, which had widespread clinical use in the past. In addition, we shall emphasize effects at the molecular and cellular levels, where relatively unambiguous conclusions may be drawn from experimental data. At the cellular level, we shall, where possible, consider experiments on isolated cells, rather than cells in a network (e.g. brain slices). (Although the latter type of studies are important, they are difficult to interpret at the present time, since anaesthetic effects on a given cell cannot usually be dissociated from those on the surrounding network. Indeed, Krnjević [32] has recently reviewed anaesthetic studies on hippocampal and neocortical neurones in isolated slices and noted the conflicting nature of the experimental results and the conclusions drawn.) At the molecular level, we shall contrast the anaesthetic sensitivities of various membrane proteins (with emphasis on ion channels) and consider briefly the question of whether or not anaesthetic effects on these proteins are mediated by effects on surrounding lipids.

ANAESTHETIC CONCENTRATIONS AND THE PROBLEM OF TEMPERATURE

First, it is necessary to consider what aqueous concentrations or gaseous partial pressures of volatile anaesthetics are relevant to clinical anaesthesia. This is because large concentrations of these agents, which are in fact solvents in their pure form, will affect almost any target (for example, membrane lipids dissolve in liquid halothane). What is important is the effects observed at or near clinical concentrations, not those observed at toxic or lethal concentrations. Since therapeutic indices for volatile agents [61, 62] are generally in the range 2 to 4, one should be suspicious of effects which are observed at concentrations greater than twice the EC_{50} value for general anaesthesia. EC_{50} values for a variety of volatile agents and animals are shown in table I.

Dose-response relationships

Anaesthetic EC_{50} values or minimum alveolar concentration (MAC) for humans and other commonly studied mammals are conventionally expressed as partial pressures. The dose-response curves are very steep: almost 90% of patients or animals are anaesthetized in the EC_{50} range 0.8–1.2. This is often assumed to reflect similarly steep responses to anaesthetic doses at the molecular level; this need not be the case, however, since whole animal responses are quantal in nature and the steep dose-response curves may be a result of nothing more than phenotypic similarity in the population of animals being studied. In fact, it is rare that steep dose-response curves are observed with isolated neuronal preparations.

Conversion between partial pressures and aqueous concentrations

When working with isolated preparations, it is often more convenient to apply known aqueous concentrations rather than gaseous partial pressures of volatile general anaesthetics. In addition, EC_{50} concentrations for general anaesthesia vary less with temperature when expressed as aqueous concentrations rather than as gaseous partial pressures. However, while the appropriate partial pressures of volatile general anaesthetics are well known,

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Anaesthetic doses. Anaesthetic targets. General anaesthesia. Ion channels. Membranes.

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TABLE I. *EC₅₀ concentrations for general anaesthesia expressed as partial pressures and as aqueous concentrations. Primary literature values are given directly. Parenthetical values were calculated using equations (1) or (2) (see text) with the primary values of water/gas partition coefficients at 37 °C in table II*

Agent	Animal	Partial pressure (% atm ≡ vol %)	Aqueous concn- tration (mmol litre ⁻¹)	Ref.
Halothane	Human	0.75	(0.21)	[53]
	Rat	1.03	(0.29)	[38]
	Mouse	0.95	(0.27)	[38]
	Dog	0.87	(0.24)	[46]
	Tadpole	—	0.23	[29]
Isoflurane	Human	1.3	(0.31)	[53]
	Rat	1.46	(0.35)	[38]
	Mouse	1.34	(0.32)	[38]
	Dog	1.28	(0.31)	[46]
	Tadpole	—	0.29	[14]
Enflurane	Human	1.7	(0.52)	[53]
	Rat	2.21	(0.68)	[38]
	Mouse	1.95	(0.60)	[38]
	Dog	2.2	(0.67)	[11]
Methoxy- flurane	Human	0.16	(0.27)	[53]
	Mouse	0.22	(0.37)	[39]
	Dog	0.23	(0.39)	[11]
	Tadpole	—	0.21	[29]
Chloroform	Human	0.5	(0.79)	[53]
	Mouse	0.84	(1.3)	[39]
	Dog	0.77	(1.2)	[11]
	Tadpole	—	1.3	[7]
Diethyl ether	Human	1.9	(9.5)	[53]
	Mouse	3.0	(15)	[39]
	Dog	3.0	(15)	[11]
	Tadpole	—	25	[14]

the corresponding aqueous concentrations are less familiar. To interconvert between the two, it is common to use either Bunsen (α) or Ostwald (λ) water/gas partition coefficients. If C_{aq} (mmol litre⁻¹) is the aqueous concentration, expressed as millimoles of anaesthetic per litre of water, in equilibrium with a gaseous partial pressure P(% atm), expressed as percentage of an atmosphere or volume percent, then the following equations apply:

C_{aq}(mmol litre⁻¹) = 0.44614αP(% atm) (1)

C_{aq}(mmol litre⁻¹) = $\frac{121.86\lambda P(\% \text{ atm})}{(273.15 + T_c)}$ (2)

where α and λ are the Bunsen and Ostwald water/gas partition coefficients, respectively, and T_c is the temperature expressed in degrees centigrade. Either equation may be used, since both give the same answer. Values of Bunsen and Ostwald coefficients for a selection of volatile agents are listed in table II for different temperatures. Note that the aqueous concentrations calculated using these tabulated partition coefficients are the concentrations in equilibrium with pure water; concentrations in experimental physiological buffers are generally 10 to 15 % smaller [50].

The problem of experimental temperature

Ideally, anaesthetic experiments on isolated tissues, neurones and membranes should be performed at the normal body temperature of the animal concerned, in which case the MAC, aqueous EC₅₀ concentration, or both, for general anaesthesia are either known or may be determined. However, for various reasons (usually of experimental convenience), anaesthetic experiments on mammalian preparations are performed frequently at temperatures considerably less than body temperatures, often at room temperature, and it is necessary to have some idea as to what concentrations are relevant to general anaesthesia. For example, in an experiment in which mammalian neurones are being studied at room temperature (20 °C), the relevant partial pressure of anaesthetic which should be used is then not the MAC for the animal at its body temperature (37 °C); a better approximation would appear to be the (perhaps hypothetical) MAC at 20 °C. In the case of halothane and dogs, Eger and colleagues [12, 48, 51] have determined MAC as a function of temperature between 28 and 43 °C. We analysed their data [15] and found they could be reasonably fit by a linear van't Hoff plot of ln(MAC) vs reciprocal absolute temperature, with a regression slope corresponding to an apparent enthalpy of binding of ΔH = -52 kJ mol⁻¹. We can express this result as a simple exponential equation:

$\frac{MAC(T_c)}{MAC(37\text{ }^\circ\text{C})} = \exp\left[\frac{-20.2(37 - T_c)}{273.15 + T_c}\right]$ (3)

where MAC(T_c) and MAC(37 °C) are the minimum alveolar concentrations (partial pressures) at

TABLE II. *Bunsen (α) and Ostwald (λ) water/gas partition coefficients. Primary literature values are given directly, with derived values in parentheses*

Agent	Formula	°C	Bunsen α	Ostwald λ	Reference
Halothane	CF ₃ CHClBr	20	1.60	(1.72)	[50]
		25	1.20	(1.31)	[50]
		30	0.92	(1.02)	[50]
		37	0.63	(0.72)	[50]
Isoflurane	CHF ₂ OCHClCF ₃	25	1.08	(1.18)	[50]
		37	0.54	(0.61)	[50]
Enflurane	CHF ₂ OCF ₂ CHClF	37	(0.69)	0.78	[1]
Methoxyflurane	CH ₃ OCF ₂ CHCl ₂	20	8.08	(8.67)	[50]
		30	5.48	(6.08)	[50]
		37	3.80	(4.31)	[50]
Chloroform	CHCl ₃	37	(3.52)	4.0	[1]
Diethyl ether	C ₂ H ₅ OC ₂ H ₅	22	(27.8)	30.0	[48]
		30	(17.8)	19.7	[48]
		37	(11.2)	12.7	[48]

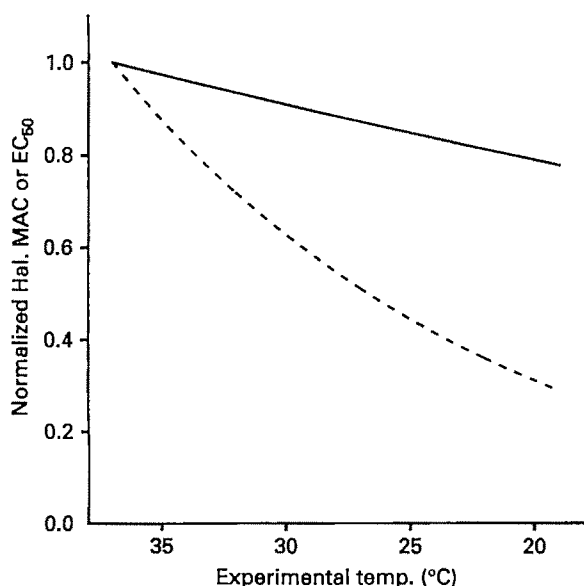


FIG. 1. EC_{50} concentrations for general anaesthesia are much less affected by temperature when expressed as aqueous concentrations than when expressed as partial pressures. The factor by which the halothane (Hal.) EC_{50} concentration for canine general anaesthesia is reduced with decreasing temperature is plotted for both partial pressures (MAC, —) and for concentrations in water (---) (the lines were drawn according to equations (3) and (4), respectively).

temperatures T_0 (in °C) and 37 °C, respectively. Thus extrapolating to 20 °C, it follows that the MAC of halothane would be only 31 % of the MAC at body temperature. It is clear that one could be seriously misled by using the body temperature MAC while working with an experimental preparation at 20 °C.

On the other hand, one can convert the halothane MAC values to aqueous EC_{50} concentrations (using data from reference [50]) to arrive at an analogous equation relating the aqueous EC_{50} at temperature T_0 to that at 37 °C:

$$\frac{EC_{50}(T_0)}{EC_{50}(37^\circ C)} = \exp \left[\frac{-4.08(37 - T_0)}{273.15 + T_0} \right] \quad (4)$$

From this it follows that the aqueous EC_{50} concentration relevant at room temperature (20 °C) is 79 % that at body temperature.

Thus the reduction in MAC partial pressures with decreasing temperature is much more severe than that for aqueous EC_{50} concentrations (see fig. 1). For example, using (see table I) $MAC = 0.87\%$ atm for halothane for dogs at 37 °C, the predicted MAC at 20 °C is only 0.27 % atm, while the corresponding aqueous EC_{50} concentrations are 0.24 mmol litre⁻¹ at 37 °C and 0.19 mmol litre⁻¹ at 20 °C.

It is clear from the above example that when working with isolated mammalian neuronal preparations at temperatures less than body temperature, where the MAC or aqueous EC_{50} for general anaesthesia at the experimental temperature is not known, a simple and good pragmatic procedure would be to use anaesthetic concentrations corresponding to aqueous EC_{50} concentrations for general anaesthesia, determined at body temperature. On the other hand, the alternative but all too common procedure of working with mammalian preparations at room temperature and using MAC

partial pressures determined at body temperature may lead to serious error. These considerations reflect the fact that the major component of the decrease in MAC with decreasing temperature, which is observed for all common volatile agents, is the very large increase in the water/gas partition coefficient. For example, as may be calculated using equation (1) and table II, a 1 % atm partial pressure of halothane in equilibrium with water produces an aqueous concentration of only 280 µmol litre⁻¹ at 37 °C but of 710 µmol litre⁻¹ at 20 °C.

VOLTAGE-GATED ION CHANNELS

One might infer from reading the literature that almost all systems which have been studied are affected by general anaesthetics, and that the mechanisms underlying general anaesthesia and its side effects are therefore likely to be hopelessly complex. This view is largely because of the fact that indeed most processes *are* affected if large enough concentrations of anaesthetics are used. However, if attention is paid to the concentrations and temperatures used, one finds that many systems are in fact relatively insensitive to clinical concentrations of these agents. This is especially true for voltage-gated ion channels.

For example, the effects of volatile anaesthetics have been studied on identified, spontaneously firing, "pacemaker" neurones from the pond snail *Lymnaea stagnalis* [19, 20]. Surprisingly, it was found that the firing activity of some neurones was unaffected at room temperature (23 °C) by 0.8 % atm halothane, which is approximately the EC_{50} partial pressure for halothane "general anaesthesia" in this animal ($EC_{50} = 0.83\%$ atm, measured [22] at 20 °C using inhibition of a withdrawal reflex). Since firing activity in these neurones involves the concerted operation of a number (at least five) of different voltage-gated channels, this finding suggests that many channels are insensitive to anaesthetics. This is illustrated by the results given in figure 2. Figure 2A shows that the size and shape of spontaneous action potentials (dependent on the activity of voltage-gated sodium, potassium and calcium channels) in insensitive neurones can be relatively unaffected by 0.80 % atm halothane, while figure 2B shows that the fast transient potassium current (" I_A "), isolated using a voltage-clamp protocol, is only marginally (< 10 %) inhibited by the same concentration of halothane.

Voltage-gated sodium and potassium channels

It has long been known [33] that clinical concentrations of volatile anaesthetics have little effect on axonal conduction (but see Raymond, Shin and Steffensen [47]), which is mediated by voltage-gated sodium and potassium channels. Haydon and Urban studied the effects of volatile agents on currents mediated by these channels in the squid axon at 6 °C and confirmed that they are remarkably insensitive to clinical concentrations of anaesthetics [24]. Although dose-response curves were not obtained, the concentrations which inhibited the peak current through the sodium channel by 50 % were found to be halothane 2.0 mmol litre⁻¹, methoxyflurane

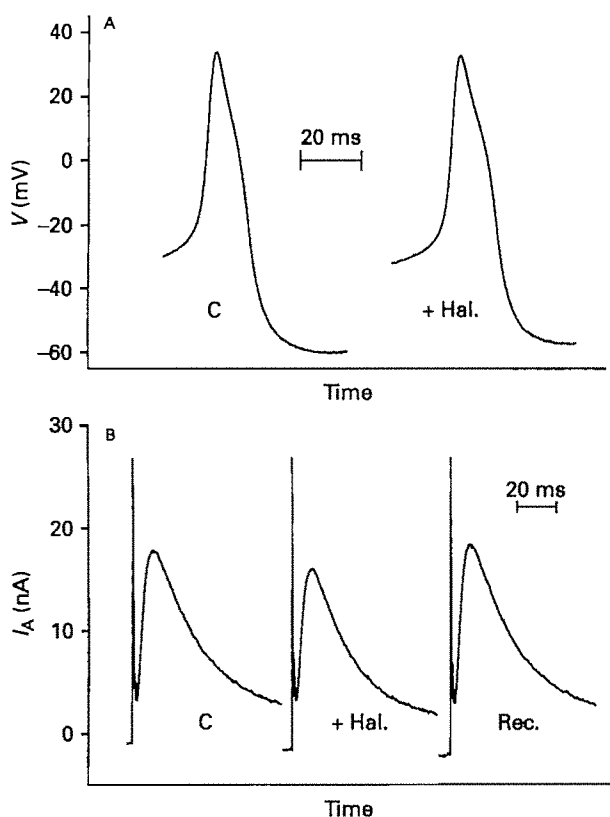


FIG. 2. Many voltage-gated channels are very insensitive to levels of volatile general anaesthetics which produce general anaesthesia. A: Spontaneous action potentials in an insensitive molluscan neurone in the absence (control (C)) and presence of 0.8% halothane (Hal.). B: Fast transient potassium currents ("I_A") in another molluscan neurone, also in the absence (C) and presence of 0.8% Hal. This current was evoked by changing the voltage-clamp potential from -100 mV to -40 mV at the times indicated by the capacitive current spikes (Rec. = recovery). (From [20], with permission.)

1.5 mmol litre⁻¹, chloroform 3.0 mmol litre⁻¹ and diethyl ether 75 mmol litre⁻¹. The "delayed rectifier" potassium current was found to be even less sensitive, being inhibited by 50% by halothane 6.4 mmol litre⁻¹, methoxyflurane 2.1 mmol litre⁻¹, chloroform 11.2 mmol litre⁻¹ and diethyl ether 150 mmol litre⁻¹. For comparison, the aqueous EC₅₀ concentrations [8] for general anaesthesia at 5 °C in another cold-blooded aquatic animal (the goldfish) are halothane 0.24 mmol litre⁻¹, methoxyflurane 0.15 mmol litre⁻¹, chloroform 0.81 mmol litre⁻¹ and diethyl ether 35 mmol litre⁻¹. Thus only the relatively polar agent diethyl ether had a reasonable potency (only a factor of two different) for inhibiting one of the currents (the sodium current) compared to its potency for producing general anaesthesia. More recently, it has been found [25] that voltage-gated sodium and potassium channels in clonal GH3 pituitary cells at room temperature are also very insensitive to halothane.

Voltage-gated calcium channels

Because of their importance in presynaptic release of neurotransmitters, secretion and cardiac function, numerous workers have investigated inhibition of voltage-gated calcium currents by volatile agents. At present there are known to be at least three major

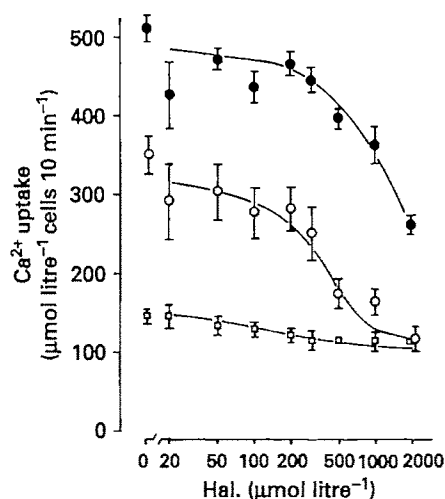


FIG. 3. Calcium uptake into bovine adrenal chromaffin cells is inhibited by halothane (Hal.) much more in response to activation of nicotinic acetylcholine receptor-channels than in response to depolarization by large concentrations of potassium. Dose-response curves are given for basal calcium uptake (□) and for calcium uptake in the presence of either 500 $\mu\text{mol litre}^{-1}$ of carbachol (○) or 77 mmol litre⁻¹ of potassium (●). The temperature was 37 °C. (From [43], with permission.)

types of calcium channel, often designated L, N and T. The channels differ in activation and inactivation voltage ranges, single-channel conductances, susceptibilities to block by cadmium and toxins, and sensitivities to dihydropyridine inhibitors [40]. Most anaesthetic studies have been performed on currents mediated by the relatively accessible dihydropyridine sensitive L channels, while almost no studies have been carried out on the less accessible (but arguably more important for the induction of general anaesthesia) N type channels which are thought to be intimately associated with neurotransmitter release sites.

Some calcium channels are extremely insensitive to inhibition by volatile agents. For example, calcium entry into bovine adrenal chromaffin cells, measured by the uptake of radioactive calcium in response to potassium-induced depolarization, is extremely resistant. Yashima, Wada and Izumi [63] found no significant effect of 2% halothane at 37 °C, while Pocock and Richards [43], also working at 37 °C, found only 50% inhibition at approximately 2 mmol litre⁻¹ of halothane (see the upper dose-response curve in fig. 3). In this latter study, using secretion of catecholamines from these chromaffin cells as a measure of calcium entry, the following (very large) IC₅₀ concentrations were obtained: halothane > 2.0 mmol litre⁻¹, isoflurane 2.2 mmol litre⁻¹, enflurane 3.2 mmol litre⁻¹ and methoxyflurane 1.2 mmol litre⁻¹. These results probably reflect anaesthetic action, predominantly on L type channels [44]. Also, Terrar and Victory [57, 58] used a voltage-clamp protocol to measure (probably L type) currents in myocytes from guinea-pig ventricle at 37 °C and found that IC₅₀ concentrations were > 1.57 mmol litre⁻¹ for halothane and > 1.78 mmol litre⁻¹ for isoflurane.

Other investigators have found calcium channels to be more sensitive to anaesthetic inhibition. Kress and colleagues [30, 31], working with the phaeo-

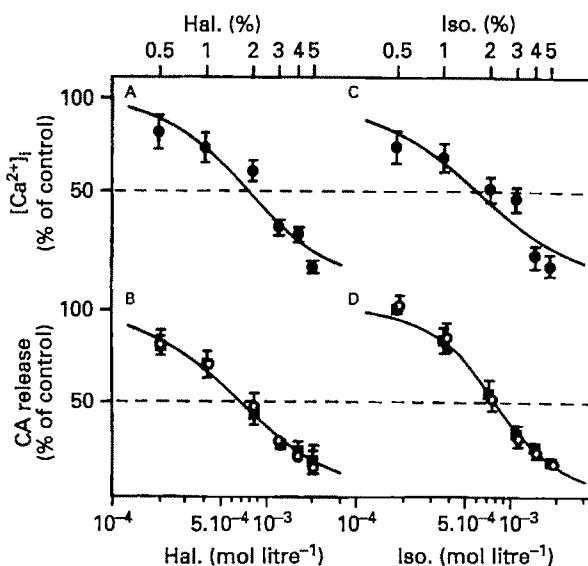


FIG. 4. The increase in peak, free intracellular Ca²⁺ ([Ca²⁺]_i) and the secretion of catecholamines (CA) in response to depolarization by large concentrations of potassium are moderately (and almost equally) sensitive to inhibition by the volatile anaesthetics halothane (Hal.) and isoflurane (Iso.) for rat pheochromocytoma PC12 cells. Dose-response curves are given for the increase in [Ca²⁺]_i (A, C) and for the secretion of dopamine (○) and noradrenaline (■) (B, D). The temperature was 32 °C. (From [30], with permission.)

chromocytoma cell line PC12 at 32 °C and measuring increases in intracellular free calcium, found that nifedipine sensitive (L type) channels appeared to be blocked by relatively modest concentrations of volatile agents (fig. 4A, C). IC₅₀ concentrations were halothane 0.75 mmol litre⁻¹, isoflurane 0.57 mmol litre⁻¹, enflurane 0.73 mmol litre⁻¹ and methoxyflurane 0.58 mmol litre⁻¹. Similar IC₅₀ concentrations were found for inhibition of catecholamine release (fig. 4B, D) from these cells. Eskinder and co-workers [13], using voltage-clamp techniques with canine cardiac Purkinje cells at 22 °C, found that IC₅₀ concentrations for both L and T type currents were approximately 0.7–0.8 mmol litre⁻¹ for halothane, 1.0–1.1 mmol litre⁻¹ for isoflurane and 1.2 mmol litre⁻¹ for enflurane. Similar IC₅₀ concentrations were found by Bosnjak, Supan and Rusch [6] for predominantly L type currents in canine cardiac ventricular cells at 22 °C. Finally, Herrington and colleagues [25], using voltage-clamp protocols with clonal GH3 pituitary cells at room temperature, found that IC₅₀ = 0.85 mmol litre⁻¹ for halothane for peak high-voltage activated currents (similar to L type currents) and IC₅₀ = 1.3 mmol litre⁻¹ for halothane for peak low-voltage activated currents (similar to T type currents). The above IC₅₀ concentrations for halothane may be compared to the predicted EC₅₀ of approximately 0.20 mmol litre⁻¹ for halothane, for causing canine general anaesthesia at room temperature. Thus even the most sensitive calcium currents measured to date are inhibited by halothane only at IC₅₀ concentrations which are three to four times the predicted EC₅₀ concentration for general anaesthesia. Nonetheless, these “sensitive” calcium currents appear to be the most sensitive of those voltage-gated ion channels which have been investigated so far.

LIGAND-GATED ION CHANNELS

In contrast to voltage-gated channels, which generally appear to be insensitive to clinical concentrations of volatile general anaesthetics, some ligand-gated channels are very sensitive. This is perhaps not surprising, in view of the fact that many of these ligand-gated channels are concentrated at synapses, which are generally considered to be the most anaesthetic-sensitive links in the transmission of nerve impulses.

Nicotinic acetylcholine receptor-channels

These are the most widely studied of all ligand-gated ion channels, and it has recently become apparent that they can be very sensitive to inhibition by volatile general anaesthetics. However, for some time this fact was obscured because it was thought [14, 64] that general anaesthetics acted by desensitizing these channels, and this required large anaesthetic concentrations. For example, figure 5 shows the dose-response curve for halothane increasing the binding of radiolabelled acetylcholine (a measure of desensitization) to receptor-rich membranes from electroplaques of *Torpedo californica* at room temperature. It is clear from the data that significant effects occurred only when the halothane concentration approached 1 mmol litre⁻¹, about four times the EC₅₀ concentration for general anaesthesia in tadpoles (see table I).

The studies of Yashima, Wada and Izumi [63] and Pocock and Richards [43] on bovine adrenal chromaffin cells have already been discussed in connection with voltage-gated calcium channels. While little sensitivity to halothane was found for radio-

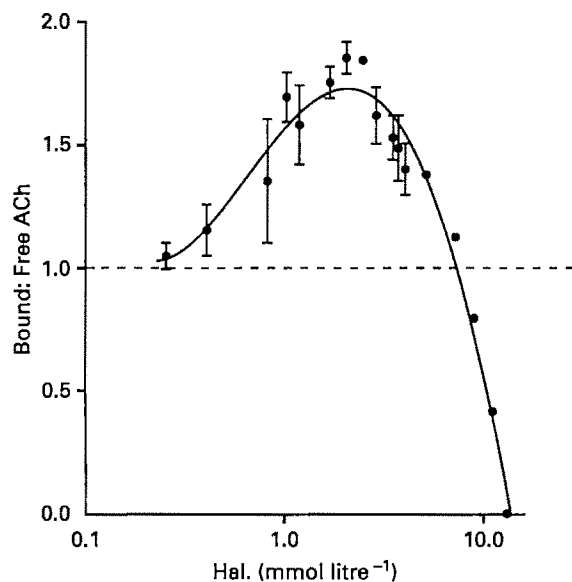


FIG. 5. The increase in binding of [³H]acetylcholine to nicotinic acetylcholine receptor-rich membranes from the electric tissue of *Torpedo californica* (a measure of receptor “desensitization”) requires large concentrations of halothane (Hal.). The ratio of bound to free acetylcholine (ACh) is plotted as a function of the aqueous concentration of halothane at room temperature. The dashed line represents the approximate bound:free ratio for ACh in the absence of anaesthetic. Note the fall-off of this ratio at extremely large concentrations of anaesthetic. (Data taken from fig. 1 of [14].)

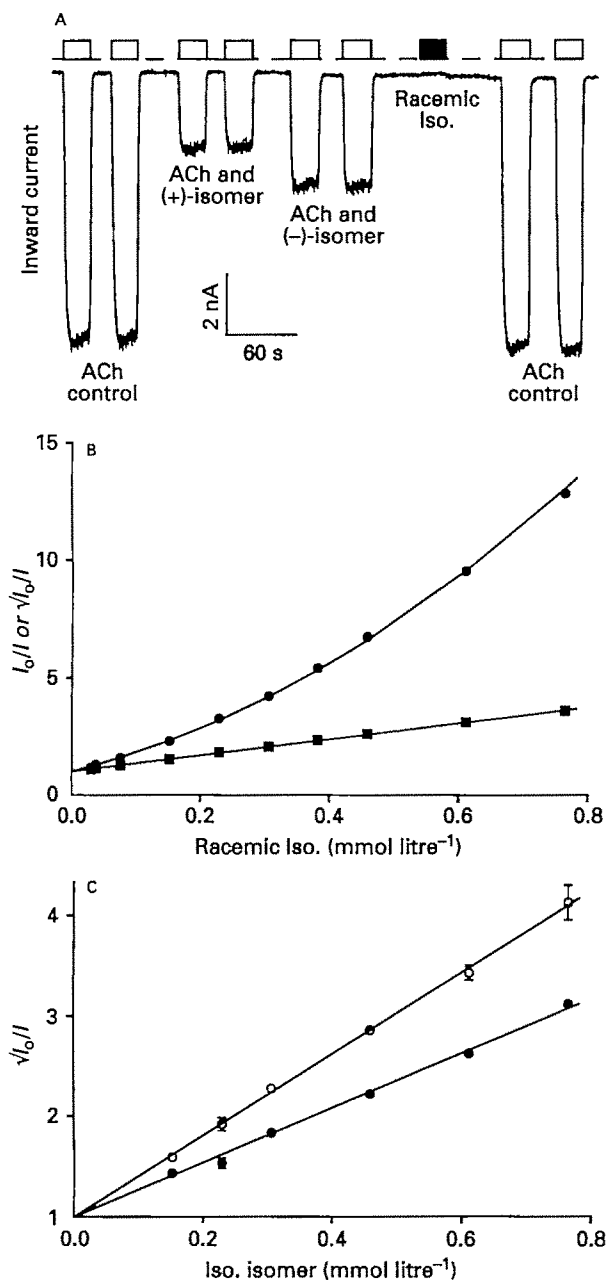


FIG. 6. The (+)-isomer of isoflurane (Iso.) is more effective than the (-)-isomer at inhibiting an inward current activated by acetylcholine (ACh) acting at a nicotinic receptor in a completely isolated identified neurone from the pond snail *Lymnaea stagnalis*. A: The inward currents caused by repeated applications (open bars) of ACh 200 nmol litre⁻¹ in the absence and presence of the anaesthetic isomers are shown, together with the background current in the presence of the racemic mixture alone (solid bar). The neurone was voltage-clamped at -70 mV. B: Dose-response relationship for inhibition by racemic isoflurane. The ratio of control to inhibited current (I_0/I) (●) and the square root of this ratio are plotted against the aqueous concentration of racemic isoflurane. The curves are the theoretical predictions of a simple binding model in which two molecules of anaesthetic may bind independently to the receptor but the presence of only one molecule is sufficient to block it. C: Dose-response relationship for each of the two optical isomers. The square root of control to inhibited current is plotted against the aqueous concentration of (+)-isoflurane (○) and (-)-isoflurane (●). According to the binding model, the reciprocals of the slopes of these lines are the anaesthetic/receptor dissociation constants. The temperature was 20 °C. (Modified from fig. 2 of [21].)

active calcium uptake in response to depolarization, greater sensitivity was found in response to the acetylcholine agonist carbachol. This is shown in figure 3 (taken from the latter study [43]), where the IC_{50} for the agonist-induced uptake (middle curve) is approximately 0.3 mmol litre⁻¹ at 37 °C. (This is almost the same value found in the earlier study [63]). A possible interpretation of these results is that small concentrations of halothane inhibit the carbachol activation of the nicotinic receptor-channel and thus the resulting depolarization, leading to reduced calcium entry, not only through the nicotinic receptor channel itself, but also through the less sensitive voltage-gated calcium channels. Going one step further and using catecholamine secretion as a measure of receptor inhibition, Pocock and Richards [43] found the following IC_{50} concentrations at 37 °C: halothane 0.30 mmol litre⁻¹, isoflurane 0.45 mmol litre⁻¹, enflurane 0.40 mmol litre⁻¹ and methoxyflurane 0.25 mmol litre⁻¹. These concentrations are very similar to EC_{50} concentrations for general anaesthesia (see table I) and suggest that the nicotinic receptor-channel in these cells is very sensitive to volatile general anaesthetics.

More direct measurements of the effects of anaesthetics on neuronal nicotinic acetylcholine receptor-channels require the use of electrophysiological voltage-clamp procedures on neurones. We [21] studied the effects of isoflurane and its two optical isomers on an acetylcholine-induced nicotinic chloride current in isolated identified neurones from *Lymnaea stagnalis*, at room temperature. This current is similar to one in *Aplysia* neurones which had previously been shown to be very sensitive to enflurane [3]. The results are shown in figure 6. Remarkably, the two optical isomers inhibited the current to different extents (fig. 6A), consistent with a direct effect on the receptor-channel rather than on the surrounding lipids (see below). The inhibition was consistent with the predictions of a simple model in which two molecules of isoflurane can independently bind to the receptor-channel but the presence of only one molecule is necessary to inhibit it (fig. 6B, C). The inhibition (dissociation) constants K_i were found to be 0.51 % atm for the (+)-isomer and 0.76 % atm for the (-)-isomer, consistent with the value of 0.60 % atm for the racemic mixture of both isomers. Because of the square dependency of the inhibition, it is easy to show that $IC_{50} = 0.414 K_i = 0.25$ % atm (~ 0.12 mmol litre⁻¹) of racemic isoflurane, similar to the value of about 0.2 % atm enflurane previously found [3] for inhibiting the analogous current from *Aplysia*. For comparison, EC_{50} partial pressures for inhibiting a withdrawal reflex in *Lymnaea stagnalis* at room temperature are 1.09 % atm for isoflurane and 1.01 % atm for enflurane [22]. Thus these molluscan nicotinic acetylcholine receptor-channels appear to be about four to five times more sensitive to anaesthetic inhibition than is the behavioural response of the whole animal.

GABA_A receptor-channels

GABA (γ -amino-*n*-butyric acid) is the major inhibitory neurotransmitter in the mammalian brain.

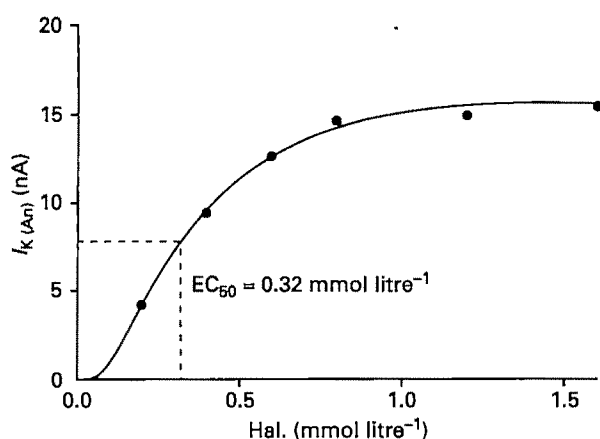


FIG. 7. Small concentrations of halothane (Hal.) activate an outward potassium current in a sensitive identified neurone from the pond snail *Lymnaea stagnalis*. This current ($I_{K(An)}$) was measured 15 s after changing the membrane potential from -80 to 0 mV. The temperature was 23°C . (Modified from fig. 3 of [19].)

Although it acts on both GABA_A and GABA_B receptors, most anaesthetic studies have been carried out on the "classical" GABA_A receptor-channel. Physiologically, binding of GABA molecules to the GABA_A receptor-channel forms a pathway for the movement of chloride ions, generally producing neuronal hyperpolarization. It has long been known that these GABA-induced chloride currents are enhanced by benzodiazepine tranquillizers, barbiturates and steroid anaesthetics, but the effects of volatile agents are only now being clarified.

Nakahiro and colleagues [42] used the whole cell patch-clamp technique to study GABA activated currents in rat dorsal root ganglion neurones in primary culture at room temperature (22°C). They found that GABA at small concentrations produced a small sustained inward current that was enhanced about three-fold by $0.86 \text{ mmol litre}^{-1}$ of halothane, $0.96 \text{ mmol litre}^{-1}$ of isoflurane and $1.89 \text{ mmol litre}^{-1}$ of enflurane. GABA at large concentrations produced a much larger inward current which rapidly desensitized to a sustained concentration; the peak current was increased only about 40% by the above concentrations of anaesthetics, while the steady state current was actually inhibited.

Lin and co-workers [34] expressed mouse cerebral cortex messenger RNA in *Xenopus* oocytes and studied GABA-induced chloride currents using the two electrode voltage-clamp technique at 25°C . At small GABA concentrations, where desensitization was not present, the GABA-induced currents were potentiated by small concentrations of volatile anaesthetics; this potentiation was diminished at greater anaesthetic concentrations. The EC_{50} concentration for halothane was approximately $0.67 \text{ mmol litre}^{-1}$, with maximum enhancement occurring at approximately $1.2 \text{ mmol litre}^{-1}$. Conversely, at large, desensitizing concentrations of GABA, halothane, isoflurane and diethyl ether had no significant effect on peak current, while enflurane actually decreased it. Jones, Brooks and Harrison [27] studied cultured hippocampal neurones at 25°C using the whole cell patch-clamp technique. Small concentration pulses

of GABA produced an inward current which was enhanced by volatile agents, with significant effects at 1 MAC concentrations. Dose-response curves did not appear to saturate up to halothane $2 \text{ mmol litre}^{-1}$, isoflurane $10 \text{ mmol litre}^{-1}$ or enflurane $3 \text{ mmol litre}^{-1}$.

Thus the potentiation of GABA-induced chloride currents by volatile anaesthetics, at least at small concentrations of GABA, would appear to be established, although it is difficult to assess its physiological relevance. For example, anaesthetic EC_{50} concentrations are considerably greater than those for general anaesthesia, but substantial potentiation does nonetheless occur at relatively small concentrations. In addition, the fact that GABA_A responses at large GABA concentrations are much less enhanced or even inhibited by volatile anaesthetics, leaves open the crucial question of how much potentiation would occur at an active synapse.

Anaesthetic-activated potassium channel

We have recently discovered [19, 20] an anaesthetic-activated potassium current in an identified "pacemaker" neurone in the right parietal ganglion of *Lymnaea stagnalis*. This current, which we call $I_{K(An)}$, was studied under two electrode voltage-clamp at room temperature. $I_{K(An)}$ may be activated by synaptic transmission in the intact ganglion or by volatile general anaesthetics, in both the intact ganglion and the completely isolated neurone. The effect of activating $I_{K(An)}$ in the spontaneously firing neurone is to silence the cell, by hyperpolarizing it to approximately 25 mV less than the threshold for action potential initiation.

$I_{K(An)}$ has been found to be activated by small concentrations of halothane, isoflurane, chloroform, and diethyl ether. The dose-response curve for halothane is shown in figure 7, where it can be seen that the $\text{EC}_{50} = 0.32 \text{ mmol litre}^{-1}$ for halothane at 23°C , which is substantially less than the $\text{EC}_{50} = 0.59 \text{ mmol litre}^{-1}$ (0.83% atm) for halothane at 20°C for "general anaesthesia" in this mollusc [22]. The current-voltage relationship may be described by the Goldman-Hodgkin-Katz constant field equation, suggesting that the channel is not voltage-gated. Indeed, voltage jump experiments showed that $I_{K(An)}$ was close to its steady state value at the earliest times (2–5 ms) at which reliable measurements could be made. In addition, $I_{K(An)}$ does not appreciably inactivate with time. Recent experiments [21] with the two optical isomers of isoflurane have shown that the (+)-isomer is about twice as effective as the (–)-isomer at activating $I_{K(An)}$, strongly suggesting a protein target. Whether this target is the channel itself or a regulatory protein remains to be established.

Calcium-activated potassium channels

Tas, Kress and Koschel [56] have recently shown that calcium-activated potassium channels may be inhibited by volatile anaesthetics in cultured rat glioma C6 cells. They studied the calcium and scorpion (*Leiurus quinquestriatus*) venom sensitive uptake and release of ^{86}Rb from these cells at 36°C . The active toxin in the scorpion venom appeared to

be charybdotoxin, a well known inhibitor of certain (usually large conductance) types of calcium-activated potassium channels. IC_{50} concentrations for halothane, isoflurane, enflurane and methoxyflurane at 36 °C were found to be very close to their MAC values for general anaesthesia in rodents. The authors make the novel and interesting suggestion that anaesthetic inhibition of these channels in glial cells may contribute to general anaesthesia by interfering with the spatial buffering of potassium ions thought to be performed by these non-neuronal cells.

ACTIVE TRANSPORT SYSTEMS

Because of the emphasis in the literature on the effects of anaesthetics on passive ion channels, the possibility that general anaesthetics may act by inhibiting "carrier" systems which actively transport ions or neurotransmitters is usually ignored. However, Tas, Kress and Koschel [55] have shown recently that small partial pressures of halothane, isoflurane and enflurane significantly inhibit the high affinity uptake of radiolabelled noradrenaline into rat pheochromocytoma PC12 cells. Using radiolabelled noradrenaline at 36 °C, the IC_{50} concentrations for inhibiting uptake were 1.8 % atm for halothane, 1.8 % atm for isoflurane, 2.4 % atm for enflurane and 0.12 % atm for methoxyflurane.

Also, an interesting earlier study [36] on the calcium ATPase of bovine cardiac sarcoplasmic reticulum, which reflects the activity of the calcium pump which accumulates calcium ions from the cardiac cytoplasm, showed that at small concentrations of free calcium, the pump activity was inhibited significantly by halothane. Working at 37 °C and measuring calcium-activated ATPase activity, they found that 1 % atm of halothane inhibited activity by $44 \pm 6\%$ at a free calcium concentration of $0.4 \mu\text{mol litre}^{-1}$. Intriguingly, the inhibition by halothane decreased with increasing free calcium concentration, consistent with competitive interactions between calcium ions and anaesthetic molecules.

Other active transport systems are not very sensitive to anaesthetic inhibition. Perhaps not surprisingly, the ubiquitous sodium-potassium pump is not inhibited by clinical concentrations of volatile agents [23]. Also, Tas, Kress and Koschel [54] have recently shown that the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transport system in rat C6 glioma cells, while sensitive to the smaller (but not the greater) *n*-alcohols, is insensitive to most volatile agents; the IC_{50} concentrations at 36 °C were $0.70 \text{ mmol litre}^{-1}$ for halothane, $10 \text{ mmol litre}^{-1}$ for isoflurane, $10 \text{ mmol litre}^{-1}$ for enflurane, $10 \text{ mmol litre}^{-1}$ for methoxyflurane and $44 \text{ mmol litre}^{-1}$ for diethyl ether.

CELLULAR RESPONSES

Volatile general anaesthetics may be selective not only at the channel level but at the cellular level. This is illustrated by studies [19, 20] on identified neurones in the pond snail. Studying neurones in an identified cluster of about 40 apparently identical neurones having endogenous firing activities, most

neurones were found to be relatively unaffected by the small concentrations of volatile agents which cause general anaesthesia. However, one identified neurone was strikingly affected, hyperpolarizing by tens of millivolts and completely losing its firing activity. This behaviour, confirmed in the isolated neurone, was found to be a result of opening of anaesthetic-activated potassium channels which were apparently absent from the surrounding neurones.

Intracellular calcium

The intracellular concentration of free calcium ions ($[\text{Ca}^{2+}]$), is one of the most important regulatory systems in the cell. It has often been suggested that anaesthetics may act by increasing $[\text{Ca}^{2+}]$, which may depress cellular activity by, for example, opening calcium-activated potassium channels and thus hyperpolarizing the cell. This increase in $[\text{Ca}^{2+}]$ could, in principle, be a result of anaesthetics causing a net release of calcium sequestered in cytoplasmic stores (e.g. microsomes, mitochondria) or by increasing the entry of calcium across the plasma membrane.

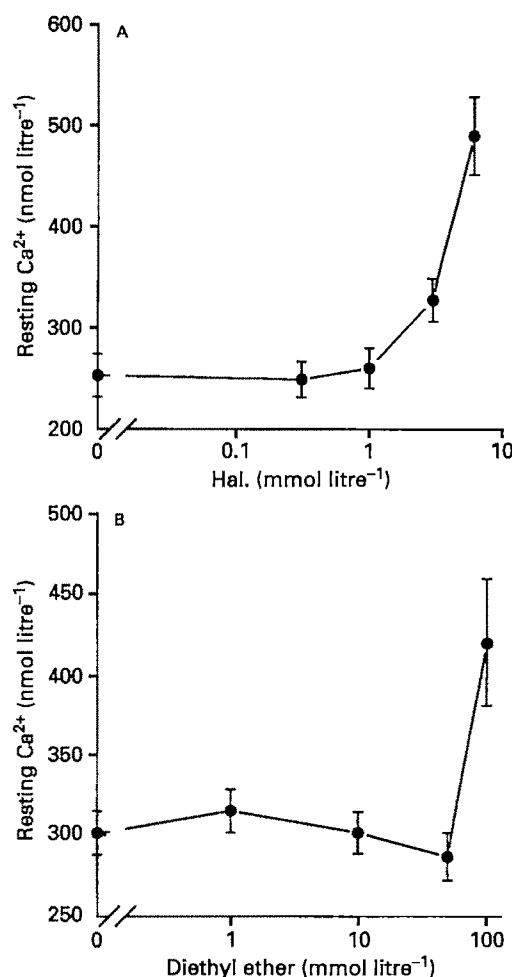


FIG. 8. Low concentrations of volatile general anaesthetics have no significant effect on the resting intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]$) of free calcium ions in synaptosomes prepared from whole mouse brain. Only at large anaesthetic concentrations was an increase in $[\text{Ca}^{2+}]$ observed. $[\text{Ca}^{2+}]$ was measured using the fluorescent Ca^{2+} indicator fura-2. Dose-response curves are given for (A) halothane and (B) diethyl ether. The temperature was 35 °C. (Data taken from fig. 1 of [9].)

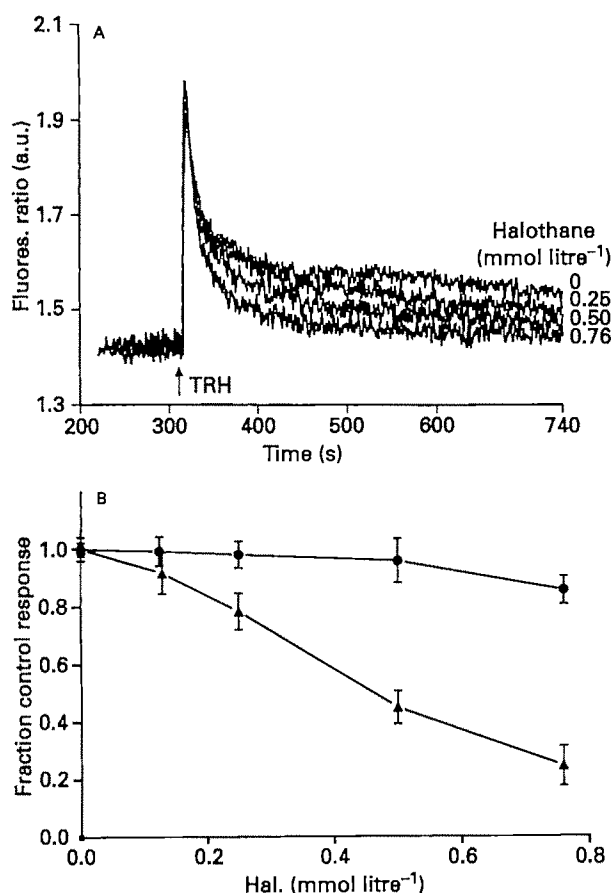


FIG. 9. Halothane (Hal.) differentially affects basal as well as peak and sustained increases in free intracellular Ca^{2+} ($[\text{Ca}^{2+}]$) in response to thyrotropin-releasing hormone (TRH) binding to clonal (GH3) pituitary cells. A: The ratio of fluorescence (Fluores.) of fura-2 at 340 and 390 nm, a measure of $[\text{Ca}^{2+}]$, is plotted as a function of time. Halothane, when present, was added 1 min before exposure of the cells to 100 nmol litre⁻¹ of TRH (arrow). Note the lack of effect of halothane on basal $[\text{Ca}^{2+}]$, the small effect on the peak increase in $[\text{Ca}^{2+}]$, and the larger effect on the sustained increase in $[\text{Ca}^{2+}]$. B: Halothane dose-response relationships for the peak (●) and the plateau (▲) responses. The halothane insensitive peak response is thought to be caused by inositol phosphate-induced release of calcium from intracellular stores, while the moderately sensitive (IC_{50} approximately 0.4–0.5 mmol litre⁻¹) sustained response is probably largely caused by influx of calcium ions via voltage-gated calcium channels. The temperature was 37 °C. (A from [52], with permission, B modified from fig. 3B of [52], with permission, with data kindly supplied by Alex Evers.)

This hypothesis has recently been tested directly in a number of cellular systems. Daniell and Harris [9] used a fluorescent Ca^{2+} indicator (fura-2) to measure $[\text{Ca}^{2+}]$ in mouse whole brain synaptosomes at 35 °C. While they found that volatile agents increased resting $[\text{Ca}^{2+}]$, this occurred only at very large anaesthetic concentrations. Figure 8 shows that there was no significant change in resting $[\text{Ca}^{2+}]$ until concentrations in excess of 1 mmol litre⁻¹ of halothane or 50 mmol litre⁻¹ of diethyl ether were used. In addition, at small anaesthetic concentrations, the increase in $[\text{Ca}^{2+}]$ caused by potassium-induced depolarization was either unaffected (by ≤ 1.0 mmol litre⁻¹ of halothane) or reduced (by 10–50 mmol litre⁻¹ of diethyl ether). Similar experiments [30, 31] with the rat pheochromocytoma cell

line PC12 showed that small concentrations of halothane, isoflurane, enflurane and methoxyflurane reduced the potassium-induced increase in $[\text{Ca}^{2+}]$ (see fig. 4 and related discussion). Puil, El-Beheiry and Baimbridge [45], working at 27 °C with cultured rat hippocampal neurones, found that 0.5–4.0 % atm isoflurane produced no significant changes in resting $[\text{Ca}^{2+}]$ while reducing the increase in $[\text{Ca}^{2+}]$ produced by application of glutamate. Finally, Stern and co-workers [52] found that the sustained increase of $[\text{Ca}^{2+}]$ caused by application of thyrotropin-releasing-hormone (TRH) to clonal GH3 pituitary cells was depressed by halothane in the range 0.25 to 0.76 mmol litre⁻¹ at 37 °C; the same concentrations of halothane had no effect on the resting $[\text{Ca}^{2+}]$ and little effect on the peak increase of $[\text{Ca}^{2+}]$ in response to TRH (see fig. 9).

In summary, it has now been shown in a variety of cellular systems that pharmacologically relevant concentrations of volatile anaesthetics have little or no effect on resting intracellular free $[\text{Ca}^{2+}]$ and if anything inhibit, rather than increase, the increase in $[\text{Ca}^{2+}]$ produced by receptor activation or membrane depolarization. These latter effects appear to reflect anaesthetic inhibition of calcium entry into cells via voltage-gated calcium channels.

The phosphatidylinositol system

In many systems, binding of agonist to a receptor activates a G protein (see below) which in turn activates a specific phospholipase C enzyme. This enzyme hydrolyses the lipid phosphatidylinositol biphosphate to yield highly charged inositol phosphates and the hydrophobic diacylglycerol (DAG). The water soluble inositol phosphates diffuse away and trigger the release of calcium ions from intracellular membrane-bound stores while DAG stays in the membrane and activates the enzyme phosphokinase C.

In the study by Stern and colleagues [52] on GH3 cells discussed above, no significant effect of halothane 0.5 mmol litre⁻¹ (at 37 °C) was found on the TRH-stimulated accumulation of inositol phosphates. This suggests that in this cell, the following proteins were unaffected by anaesthetic: the TRH receptor, the hormone-stimulated enzyme phospholipase C and the G protein linking it to the receptor. Bazil and Minneman [4] also looked at the effect of halothane on the accumulation of inositol phosphates in slices of rat cerebral cortex both at rest and in response to noradrenaline, carbachol, histamine, and 5-hydroxytryptamine. At 37 °C, 1.25 % halothane (0.35 mmol litre⁻¹) had no significant effect on either basal or agonist-induced accumulation of inositol phosphates. Thus these studies, coupled with the numerous observations listed above of no change in resting intracellular free calcium concentrations, suggest that the inositol phosphate branch of the phosphatidylinositol second messenger system is relatively refractory to clinical concentrations of volatile general anaesthetics. These results also suggest that production of DAG via the second branch of this system may also be refractory, although anaesthetics may well affect the activity of phosphokinase C.

G proteins

The first intracellular step following the agonist-induced activation of many receptors takes place via an intermediate called a G protein (guanine nucleotide binding protein) [5, 26, 49]. Indeed, about 80 % of all known receptors are coupled to G proteins [5]. G proteins are implicated in the general anaesthetic actions of α_1 adrenergic agonists [37] and are obvious *a priori* candidates for volatile anaesthetic targets.

In a recent study, Anthony, Dennison and Aronstam [2] investigated the effects of isoflurane, enflurane and chloroform on the binding of various ligands to muscarinic acetylcholine receptors in rat brain stem at room temperature, in both the presence and absence of guanine nucleotides. They found that these anaesthetics increased the binding of antagonist (but not agonist) and decreased the ability of guanine nucleotides to convert receptors to a state of low affinity for agonists. Unfortunately, the experiments were carried out at very large (2 % atm) anaesthetic concentrations for room temperature studies (see fig. 1 and table I). Bazil and Minneman [4], working with rat brain cortical slices at 37 °C and using a clinically relevant concentration of halothane (1.25 % atm), observed no effects of halothane on agonist or antagonist binding to muscarinic acetylcholine receptors or to α_1 , α_2 and beta adrenergic receptors or on the ability of guanine nucleotides to reduce agonist binding affinity. In addition, they found no effect of this concentration of halothane on noradrenaline-stimulated cAMP accumulation or inositol phosphate accumulation in response to a number of agonists. Finally, as mentioned above [52], the G protein linking the TRH receptor to the hormone-stimulated phospholipase C enzyme in GH3 cells appears to be unaffected by halothane 0.5 mmol litre⁻¹ at 37 °C.

Overall, the little work that has been done in this area suggests that although effects may occur at large anaesthetic concentrations, several G proteins are relatively unaffected by small clinical concentrations of volatile agents.

Cyclic nucleotides

There are conflicting reports on the *in vivo* effects of volatile anaesthetics on cyclic AMP (cAMP) concentrations, but more general agreement concerning effects on cyclic GMP (cGMP) concentrations. In an early study, Nahrwold, Lust and Passonneau [41] administered partial pressures of halothane to mice and subsequently used radio-immune assays to measure cAMP and cGMP concentrations in cerebral cortex, cerebellum and dorsal spinal cord. At 1.4 % atm (MAC = 0.95 % atm in mice), there were significant (greater than three-fold) changes in cGMP concentrations in the cerebral cortex (increase) and in the cerebellum (decrease) but no change in the spinal cord; however, there were no significant changes in cAMP in any of the three regions at this concentration of halothane. In a similar study with mouse cerebellum, Triner and co-workers [59] also reported that 0.5–1.5 % atm halothane decreased substantially (by

about two-fold) cGMP concentrations; comparable but more substantial decreases were found by Vulliemoz and colleagues [60] using enflurane. These decreases in cGMP were interpreted as being a consequence of anaesthetic-induced decreases in Purkinje cell activity [59, 60].

In contrast to the negative results of Nahrwold, Lust and Passonneau [41] with cAMP in mice, a number of investigators [10, 28, 35] have reported anaesthetic stimulated increases in rat brain cAMP. For example, MacMurdo and colleagues [35] found dose-related increases (about two- to three-fold at the largest doses) in whole rat brain cAMP concentrations subsequent to anaesthetizing rats with 1.5–2.0 % atm halothane and 2.5–7.5 % atm diethyl ether, respectively.

More recently, in an *in vitro* study, Bazil and Minneman [4] reported that 1.25 % halothane at 37 °C did not affect significantly either basal or noradrenaline-induced cAMP accumulation in slices of rat cerebral cortex. The disparity between the cAMP results of this *in vitro* study and most of the above *in vivo* studies raises the possibility that *in vivo* cyclic nucleotide concentrations reflect predominantly neuronal activity and thus may be simply a consequence of, rather than a cause of, general anaesthesia.

LIPID OR PROTEIN TARGETS?

Until recently, it was believed widely that general anaesthetics act by disrupting lipid interactions in cell membranes. This led to the belief that general anaesthetics (and, in particular, volatile agents and gases) must act indiscriminately and in a "physico-chemical" manner. The general idea was that anaesthetic molecules first dissolve in the lipid regions of membranes, changing their physical properties (such as fluidity or volume). This in turn was thought to modulate the activity of integral membrane proteins, such as receptors and ion channels, which were embedded in the lipid regions. Thus lipids were thought to be the primary targets and membrane proteins the secondary targets.

During the past 15 years, however, increasing evidence has favoured the view that proteins and not lipids are the primary targets of anaesthetic action (for reviews, see [15, 18]). This evidence has been of three types. (1) Structural and dynamic studies on pure lipid bilayers have shown that although large anaesthetic effects can be observed at large anaesthetic concentrations, the effects observed at EC₅₀ concentrations for general anaesthesia are usually barely measurable. Moreover, such changes as do occur at surgical EC₅₀ concentrations may almost always be mimicked by very small changes (< 1 °C) in temperature, comparable to temperature variations within the normal physiological range. (2) It was discovered [16] that a pure lipid-free protein (firefly luciferase) could not only bind a diverse range of anaesthetic agents but was inhibited at IC₅₀ concentrations which were essentially identical to those which produce general anaesthesia. Furthermore, "cut-off" effects found for inducing general anaesthesia when ascending homologous series of

alcohols and alkanes were mimicked by this enzyme [17]. (3) Recent experiments [21] with the two optical isomers of isoflurane have shown that although they have identical effects on pure lipid bilayers, they act stereoselectively on particularly sensitive neuronal ion channels (see fig. 6). Such discrimination is characteristic of direct interactions with proteins.

CONCLUSIONS

If attention is paid to anaesthetic concentrations, taking account of experimental temperatures, volatile general anaesthetics are found to be selective in their actions at the molecular and cellular levels. Most voltage-gated ion channels are very insensitive to these agents, although certain voltage-gated calcium channels are inhibited significantly at surgical concentrations. Ligand-gated ion channels, on the other hand, may be much more sensitive and activated or inhibited substantially at clinical concentrations. Most of the few second messenger systems which have been investigated so far are relatively insensitive, with the possible exception of those involving cyclic nucleotides. Volatile anaesthetics appear to act selectively on particularly sensitive proteins rather than on the lipid bilayer regions of cell membranes.

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ADRENOCEPTORS AS MODELS FOR G PROTEIN-COUPLED RECEPTORS: STRUCTURE, FUNCTION AND REGULATION

D. A. SCHWINN

Catecholamines, either endogenous hormones or a variety of exogenous synthetic pharmacological agents, bind to adrenoceptors to mediate their ultimate clinical effects [21]. Since adrenoceptors mediate many cardiovascular responses, pharmacological stimulation and inhibition of adrenoceptors may be useful to the anaesthetist. Ahlquist described originally two adrenoceptor subtypes (alpha and beta) in 1948 [1], but for many years four "classic" adrenoceptor subtypes (alpha₁, alpha₂, beta₁ and beta₂) have guided our understanding of human physiological responses and clinical administration of catecholamine drugs. Recently, genes encoding nine distinct adrenoceptor subtypes (alpha_{1A}, alpha_{1B}, alpha_{1C}, alpha_{2C1}, alpha_{2C2}, alpha_{2C10}, beta₁, beta₂ and beta₃), have been discovered. The elucidation of new adrenoceptor subtypes has already facilitated the search for, and development of, adrenoceptor subtype selective agonists and antagonists.

Adrenoceptors are members of the larger guanine nucleotide binding protein (G protein)-coupled receptor super family [4, 27, 33, 36]. In general, G protein-coupled receptors are excitable proteins, located in the cell membrane, coupled via intermediary G proteins to effector systems. Since activation of G protein-coupled receptors results in desired (or occasionally undesired) clinical effects, these receptor systems are extremely important in clinical medicine. However, not all clinically important physiological responses are mediated via G protein-coupled receptors. Thus in order to provide a framework in which to understand the importance of G protein-coupled receptors in clinical medicine and anaesthesia, this review begins with a basic overview of various methods of cell communication, with emphasis on excitable transmembrane proteins. Because adrenoceptors have been studied intensely (in terms of receptor structure, function and regulation), they provide good models for studying mechanisms of action of G protein-coupled receptors in general. Therefore, after introducing general concepts of cell communication, this review will focus primarily on adrenoceptors as models of G protein-coupled receptors. Whenever possible, only review articles (or landmark discoveries) will be cited

in order to facilitate a simplified approach while simultaneously providing in depth coverage of important concepts and topics.

EXCITABLE TRANSMEMBRANE PROTEINS

Cells communicate with their environment in many different ways. Three general methods of cell communication and effecting cellular (and ultimately clinical) responses are mediated via cytoplasmic receptors, stimulation or inhibition of enzyme systems, and excitable transmembrane proteins (fig. 1). Cytoplasmic receptors may be represented by steroid hormone receptors. Since steroid ligands are lipophilic (non-charged, and hence soluble in lipid), they cross the lipid bilayer of the cell membrane easily and interact directly with steroid receptors located in the cytoplasm. In addition to cytoplasmic receptors, various enzyme systems are also located in the cytoplasm; these systems may be either associated closely with the plasma membrane or present in other intracellular locations. Inhibition or stimulation of enzymes may be important in medical therapeutics, as seen with drugs such as amrinone and milrinone, which inhibit type III (cAMP) phosphodiesterase. However, in spite of the above examples of pharmaceutical agents acting via cytoplasmic receptors and cellular enzyme systems, the vast majority of current clinically used drugs and endogenously secreted hormones mediate their effects via excitable transmembrane proteins.

Three basic types of excitable transmembrane proteins can be described (fig. 2): voltage sensitive

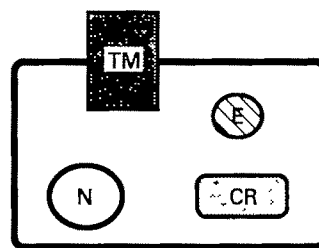


FIG. 1. Three general methods of cell communication which effect cellular (and ultimately clinical) responses include cytoplasmic receptor (CR), stimulation or inhibition of enzyme systems (E), and excitable transmembrane proteins (TM). The location of each system is illustrated schematically in relation to the cell nucleus (N) and cell membrane.

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KEY WORDS

Ion channels. Membranes. Receptors: adrenergic, function, structure.

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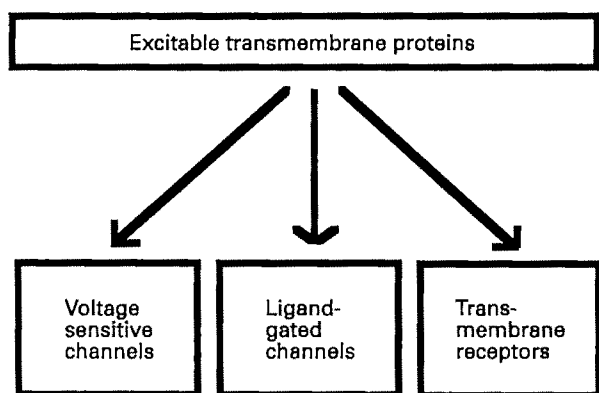


FIG. 2. Three types of excitable transmembrane proteins are important in mediating cell responses to endogenous hormones or exogenous drugs. These include voltage sensitive channels, ligand-gated channels and transmembrane receptors.

ion channels, ligand-gated ion channels and transmembrane receptors. Voltage sensitive ion channels open and close depending on cell membrane voltage and are represented by classic ion channels, such as sodium, chloride, potassium and calcium channels. Voltage sensitive ion channels are reviewed in detail elsewhere in this issue and so will not be described further in this review. The second type of clinically important excitable transmembrane proteins include ligand-gated ion channels, such as nicotinic cholinergic receptors and amino acid receptors (including GABA_A and N-methyl-D-aspartate (NMDA) receptors). Ligand-gated ion channels are receptor-ion channel complexes in which the ion channel is an integral part of a larger and more complex transmembrane protein (fig. 3). Interestingly, ligand-gated ion channel complexes are modulated frequently by more than one ligand, ion, or both. For example, GABA_A receptors bind GABA, benzodiazepines, barbiturates and ethanol, while NMDA receptors bind NMDA, phencyclidine (for which ketamine is an analogue), glycine and various ions. For further information regarding ligand-gated ion channels, the reader is referred to a recent review [4].

The third general type of excitable transmembrane proteins involved in cell communication and activity are transmembrane receptors. Cell membrane receptors may be defined as excitable transmembrane proteins which interact selectively with extracellular compounds (either endogenous hormones or exogenous drugs) to initiate a cascade of biochemical changes which lead to the ultimate physiological effect. Since transmembrane receptors are located in the lipid cell membrane, they are able to bind hydrophilic (water soluble, and therefore frequently charged) ligands located in the extracellular space. Thus many hydrophilic hormones and drugs do not have to cross the lipid bilayer to interact with the cell. However, this process then necessitates a mechanism by which transmembrane receptors notify the cell of receptor occupancy by ligands. Such a process is frequently referred to as signal transduction. Since signal transduction is reviewed separately in this symposium, only a brief overview will be given here.

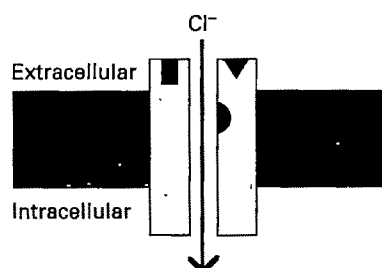


FIG. 3. Ligand-gated ion channels are receptor-ion channel complexes in which the ion channel is an integral part of a larger and more complex transmembrane protein. The ion channel is depicted as two thin rectangles traversing the cell membrane (shaded boxes). Black squares, triangles and half circles represent binding sites for ligands and ion modulators of the channel (see text for details and examples).

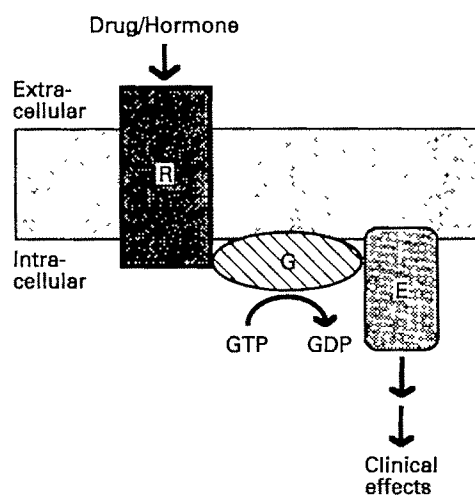


FIG. 4. Transmembrane receptors (R) are located in the cell membrane and bind drugs or hormones on the extracellular surface. Agonist bound receptors then interact with intermediary guanine nucleotide proteins (G proteins). With the energy provided by hydrolysis of GTP to GDP, activated G proteins are then able to interact with effector systems (E), ultimately leading to clinical effects.

Many receptors communicate agonist occupancy via guanine nucleotide proteins (G proteins) [15, 19]. Figure 4 shows a schematic diagram of a model G protein-coupled receptor system. An extracellular ligand (either an endogenous hormone or exogenous drug) couples to the transmembrane receptor. Thus activated, the receptor is able to interact with the intermediate G protein. Hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) provides the energy for the activated G protein to then interact with the effector molecule (either an enzyme system or ion channel) to mediate the final cascade of biological reactions within the cell which ultimately lead to the observed biological effects. Many different transmembrane receptors are part of the larger super family of G protein-coupled receptors. Examples of clinically important G protein-coupled receptor systems used currently and possibly in the future by the anaesthetist include adrenergic, muscarinic cholinergic, opioid, serotonin, dopamine, endothelin, atrial natriuretic, cannabinoid and cholecystokinin receptors. Since adrenoceptors have been studied intensively and

nine distinct subtypes have now been cloned, adrenoceptors provide good models for G protein-coupled receptors. The rest of this review will be devoted to adrenoceptors, including their subtype classification, classical pharmacology and molecular pharmacology. It is important to keep in mind, however, that many of the structural features of adrenergic receptors can be generalized to other G protein-coupled receptors.

ADRENOCEPTORS

In 1948, Ahlquist identified two types of receptors which he denoted alpha and beta [1]. In 1967, Lands and colleagues further distinguished two beta adrenoceptor subtypes: beta₁ and beta₂ [25, 26]. During the 1970s, four adrenoceptor subtypes became apparent: alpha₁, alpha₂, beta₁ and beta₂. Each of these receptors couples to distinct signal transduction pathways and will be referred to as the "classic" adrenoceptor subtypes in this review (as most of the actions of clinically available catecholamine agonists and antagonists are thought of in terms of these four subtypes). During the late 1980s and early 1990s, genes encoding nine distinct adrenoceptor subtypes were cloned, so it is known that at least nine distinct adrenoceptor subtypes exist: these include alpha_{1A}, alpha_{1B}, alpha_{1C}, alpha_{2C2}, alpha_{2C4}, alpha_{2C10}, beta₁, beta₂ and beta₃ adrenoceptors [12, 13, 16, 17, 22, 24, 29, 30, 35, 37, 38, 46]. In terms of physiological cardiovascular responses, alpha₁ adrenoceptors are located on postsynaptic membranes and mediate vasoconstriction. Alpha₂ adrenoceptors are located both presynaptic where they inhibit the release of noradrenaline at the nerve terminal, and postsynaptic where they also mediate vasoconstriction. Beta₁ adrenoceptors are thought classically to mediate cardiac effects, such as increased heart rate and myocardial inotropy, while beta₂ adrenoceptors mediate smooth muscle dilation such as bronchodilation in the lungs and vasodilation in peripheral vessels. These classic physiological descriptions are now known to be incomplete. For example, it is clear that beta₂ adrenoceptors are also located in human myocardial tissue where they mediate increased myocardial inotropy as well as possibly mediating increases in heart rate. In spite of these limitations, classic descriptions of adrenoceptor subtype-mediated physiological effects have been useful in that they provide an effective framework in which to use therapeutic agents currently available clinically.

In addition to describing adrenoceptors in terms of end-organ physiology, each subtype can be characterized in terms of classical pharmacology experiments, specifically agonist potency series and selective agonists and antagonists. The agonist potency series for alpha₁ adrenoceptors is as follows: adrenaline ≥ noradrenaline ≥ phenylephrine ≥ isoprenaline. Interestingly, the agonist potency series for alpha₂ adrenoceptors is identical to that for alpha₁ adrenoceptors. This is one of the reasons that identification of alpha adrenoceptor subtypes (specifically alpha₁ and alpha₂) was difficult historically. Instead, definitive characterization of

alpha₁ and alpha₂ adrenoceptors came with the discovery of selective antagonists such as prazosin (alpha₁ adrenoceptor), also idazoxan and yohimbine (alpha₂ adrenoceptor). Selective agonists such as phenylephrine (alpha₁), clonidine and dexmedetomidine (alpha₂) have since been discovered. Beta adrenoceptors have the following agonist potency series: beta₁, isoprenaline > adrenaline ≥ noradrenaline > phenylephrine; beta₂, isoprenaline > adrenaline > noradrenaline ≥ phenylephrine. In general, subtle differences between adrenaline and noradrenaline affinity discriminate beta₁ from beta₂ adrenoceptors. Relatively selective antagonists have been found for beta adrenoceptors, for example metoprolol and betaxolol (beta₁) and pindolol (beta₂). A selective agonist for beta₁ adrenoceptors is not yet available, but salbutamol is a clinically available selective beta₂ adrenoceptor agonist.

In addition to physiological end-organ response and ligand binding, adrenoceptor subtypes also utilize different G proteins and second messenger pathways (fig. 5) [4, 15, 19, 21, 27, 33, 36]. In general, beta adrenoceptors (once bound with agonist) interact with the stimulatory G protein, G_s, to activate the enzyme adenylyl cyclase [21]. Stimulation of adenylyl cyclase results in the production of cAMP which ultimately activates various protein kinases within the cell to cause phosphorylation of a cascade of proteins which result in the ultimate physiological response [9, 39]. Alpha₂ adrenoceptor subtypes couple to an opposing pathway. Once bound by agonists, alpha₂ adrenoceptors couple to the inhibitory G protein, G_i, to inhibit the activity of adenylyl cyclase [34]. This decreases production of cAMP in the cell. In fact, in order to study alpha₂ adrenoceptor activation, it is necessary frequently to stimulate the adenylyl cyclase moiety directly with forskolin and monitor inhibition of forskolin stimulation of adenylyl cyclase activity. Interestingly, alpha₁ adrenoceptors activate a completely different pathway. Agonist activation of alpha₁ adrenoceptors enables receptor coupling to the newly described G protein G_q [6, 11], which then activates the enzyme phospholipase C. Activation of phospholipase C results in the hydrolysis of membrane phospholipids to major products, inositol triphosphate (IP₃) and diacylglycerol (DAG) [5]. IP₃ then binds to its own receptor located on intracellular membranes to cause the release of intracellular calcium from non-mitochondrial stores, particularly the sarcoplasmic reticulum. DAG activates the enzyme protein kinase C, which then modulates this process [32].

It is important to understand signal transduction pathways for each major adrenoceptor subtype in order to understand the mechanism of action of clinically important drugs. This becomes particularly important when two drugs affecting a given signal transduction pathway at different effector points are administered simultaneously. For example, in acute heart failure during cardiac surgery, administration of a beta adrenoceptor agonist, such as adrenaline, is clinically efficacious. Since activation of myocardial beta adrenoceptors by adrenaline leads to increased intracellular cAMP,

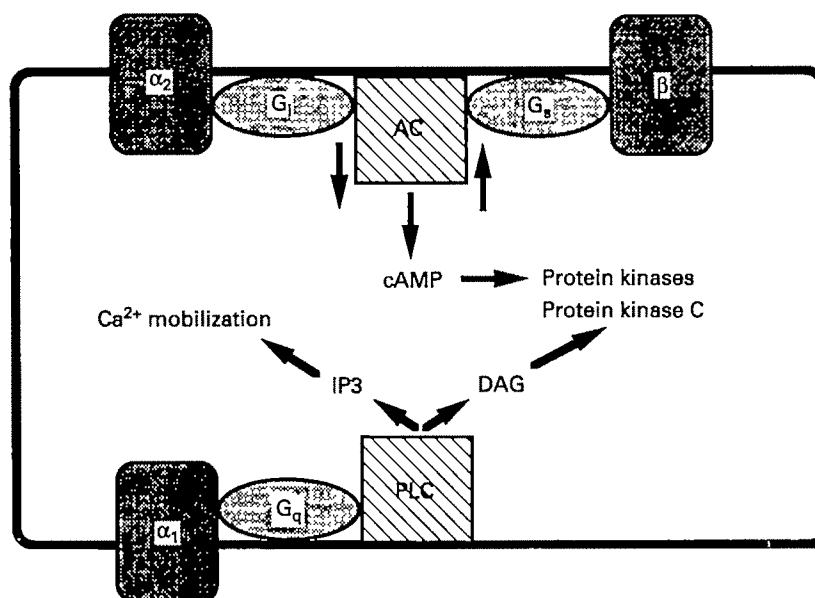


FIG. 5. Adrenergic receptor subtype second messenger systems. Receptors (α_1 (α_1), α_2 (α_2) and β) interact with intermediary guanine nucleotide proteins (G proteins, G_i , G_s and G_q , respectively) and ultimately second messenger systems (adenylyl cyclase (AC), phospholipase C (PLC)) to activate a cascade of biochemical reactions and enzyme systems (protein kinases, inositol triphosphate (IP₃), diacylglycerol (DAG) and calcium (Ca^{2+}) mobilization) (see text for details).

addition of a phosphodiesterase inhibitor, such as amrinone (which inhibits the breakdown of cAMP), will augment cAMP concentrations in myocardial cells. This ultimately leads to additive (and potentially synergistic) effects of increased myocardial inotropy. Hence, by understanding receptor systems and their modulation in disease states, clinical effects of drug administration may often be predicted for a given patient.

MOLECULAR PHARMACOLOGY OF ADRENOCEPTORS

Molecular pharmacology focuses on understanding receptor structure as it relates to pharmacological properties and ultimate function and regulation. Included are concepts such as general receptor structure, elucidation of regions of the receptor important for ligand binding, second messenger coupling and desensitization or regulation. However, in order to understand the molecular pharmacology of a given receptor, it is important to isolate the receptor from others. This process is made difficult by the fact that many receptors (and receptor subtypes) are present simultaneously in a given human cell or tissue. Therefore, in order to study the properties of a single adrenoceptor subtype, it is important to isolate the receptor from other receptors in the same tissue. One of the classic methods used to isolate an individual receptor subtype is to purify the receptor protein to homogeneity and then reconstitute the receptor in an artificial membrane system [8, 33]. This enables each component of the receptor system to be studied in detail.

The first step in purifying a single transmembrane receptor is to remove the lipid cell membrane; this is frequently accomplished using a detergent such as digitonin. The next steps relate to purification of the desired receptor from other receptors present in the tissue. A major advance in receptor purification over

the past 15 years is the concept of affinity chromatography. An affinity chromatography column is a solid support attached to long side chain molecules ending with a ligand congener which binds the receptor. Therefore, once solubilized, receptors may be separated by affinity chromatography. Only the receptor which binds the ligand attached to the column should stick, while other receptors remain free. After repeated washes, isolated receptor can be eluted from the column with a greater concentration of ligand or salt, resulting in a receptor which is highly purified. Frequently, if complete homogeneity is required, then several columns can be used.

Receptor purification to homogeneity has been accomplished over the past decade in many receptor systems, enabling rapid advances in the field of molecular pharmacology. However, while receptor purification has provided important steps forward in studying receptors, this process may be long and tedious, sometimes taking days to weeks to purify enough receptor for a single experiment. Introduction of molecular biology techniques has greatly simplified this process and therefore is presented briefly below.

Molecular biology is based on the fact that every protein in the body, albeit an enzyme, structural element or transmembrane receptor, is encoded in deoxyribonucleic acid (DNA). The nucleotide sequence present in DNA which encodes an entire protein is called a gene. Since DNA is found in the cell nucleus and the building blocks of proteins (i.e. amino acids) are assembled in the cytoplasm, an intermediate molecule is necessary to transmit information from the nucleus to the cytoplasm. Messenger ribonucleic acid (mRNA) is such an intermediary molecule; once information is transcribed from DNA to mRNA, mRNA then moves to the cytoplasm where it interacts with

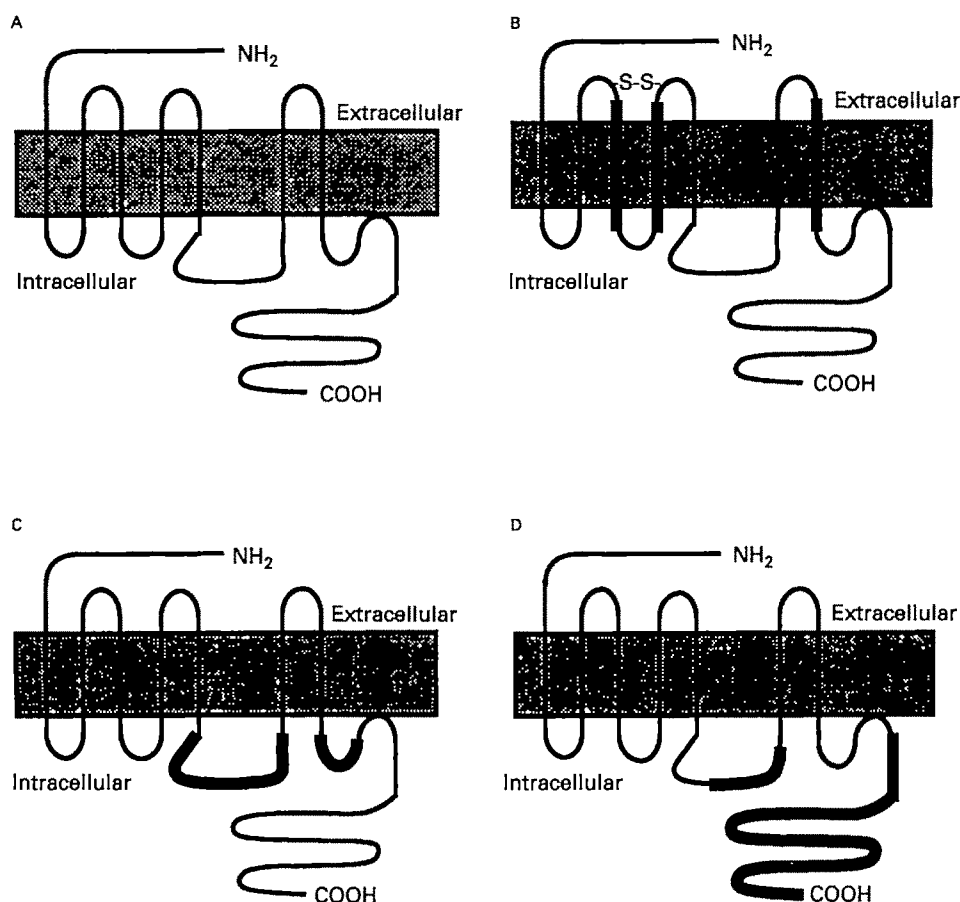


FIG. 6. Overall structure of adrenergic receptors in the cell membrane (A). This general seven transmembrane motif is common to all G protein-coupled receptors known to date. An extracellular amino terminus, three extracellular loops, three intracellular loops and an intracellular carboxyl terminus are apparent. Since the initial portion of the carboxyl terminus is anchored to the lipid membrane via palmitoylation at a critical cysteine residue, a fourth intracellular loop is actually formed. Regions of the receptor important in ligand binding, G protein coupling and desensitization are shown in B, C, and D, respectively (see text for details).

ribosomal RNA and transfer RNA which make up the "factories" where proteins are built. The process of taking encoded information from mRNA and making a protein is called translation. Thus information is transcribed from DNA to RNA and then translated into proteins. The protein is then mobilized to its appropriate location. Interestingly, the human genome frequently contains non-translated sequences surrounding each gene which are thought to modulate transcription rate, ultimately controlling the concentration of final encoded protein.

Using classical molecular biology techniques [31, 45], genes encoding nine adrenoceptor subtypes have been discovered [12, 13, 16, 17, 22, 24, 29, 30, 35, 37, 38, 46]. While the first adrenoceptor gene to be isolated and sequenced encoded the β_2 subtype, we now know that at least three α_1 , three α_2 , and three β adrenoceptor subtypes exist. Incorporation of adrenoceptor genes into cells not normally expressing these receptors results in cell lines containing individual adrenoceptor subtypes, enabling testing and elucidation of the signal transduction properties of each individual receptor subtype. Purification (both partial and completely to homogeneity) may be accomplished more easily from cell lines expressing large adrenoceptor subtype concentrations (i.e. 2–5 pmol receptor/mg total pro-

tein), while tests of physiological function may be performed in cells expressing smaller, more physiological concentrations of receptor protein (i.e. 100–250 fmol/mg total protein). Because of these molecular biology techniques, a great deal of progress has been made in understanding how receptor structure relates to physiological function. Therefore, the rest of this review will summarize general structural properties of adrenoceptors, specifically regions of adrenoceptors involved in ligand binding, G protein-coupling and regulation. More in depth analysis of these topics may be found in more detailed general reviews [27, 33, 36], as well as specific reviews cited in each section below.

General structure

One of the most striking features of adrenoceptors (and G protein-coupled receptors in general) is the seven transmembrane motif (fig. 6A). The presence of seven stretches of 20–25 lipophilic amino acids suggests that the receptor protein winds in and out of the cell membrane seven times, creating an extracellular amino terminus, intracellular carboxyl terminus, three small extracellular loops and three intracellular loops [27]. Of note, the third intracellular loop is larger and potentially more important in signal transduction than other intracellular loops. It is important to note that this overall structure has

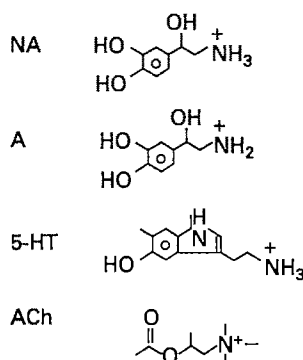


FIG. 7. Chemical structure of catecholamine ligands (adrenaline (A) and noradrenaline (NA)) compared with serotonin (5-HT) and acetylcholine (ACh).

been confirmed using various biochemical experiments and more recently with anti-peptide antibodies to every major region of the β_2 adrenoceptor receptor [27, 33, 36]. In comparing the overall structure of individual adrenoceptor subtypes, it is important to note that while the same seven transmembrane motif holds, some overall structural differences do occur. For example, α_1 adrenoceptors contain a very long intracellular carboxyl terminus, α_2 adrenoceptors a very short intracellular carboxyl terminus but extremely long third intracellular loop, while β adrenoceptors are intermediate between these two extremes.

When comparing individual amino acid sequences between adrenoceptor subtypes, another general feature is observed. If the identity of amino acids between two adrenoceptor subtypes (i.e. α_1 and β_2) is considered, matching amino acids are found to be concentrated in the transmembrane regions. Interestingly, when comparing transmembrane amino acid identity between two adrenoceptor subtypes within the same subfamily (i.e. α_{1B} and α_{1C} adrenoceptors), transmembrane amino acid identity is as great as 72–75% [37]. Comparing amino acids between adrenoceptor subtypes in different subfamilies (i.e. α_1 vs α_2 , α_1 vs β), transmembrane amino acid identity decreases to approximately 42–45% [27, 33, 36]. Transmembrane amino acid identity between adrenoceptors and another G protein-coupled receptor system (i.e. muscarinic cholinergic receptors) is approximately 35%. Since ligand binding properties are related most closely within a given subfamily of adrenoceptors, this information suggests that the transmembrane region may be important in ligand binding.

Ligand binding

In order to understand the structural requirements for ligand binding to receptors, it is important to consider the overall structure of catecholamine ligands. Figure 7 shows the chemical structure of the catecholamines adrenaline and noradrenaline compared with serotonin and acetylcholine. Adrenaline and noradrenaline contain a catecholamine ring moiety, hydroxyl groups at both the catecholamine ring and beta hydroxy positions, as well as a protonated amine group. In contrast, serotonin

contains a catecholamine ring but no hydroxyl group in the beta hydroxy position, and the protonated amine group is further from the catechol ring. Acetylcholine has a different overall structure. In order to bind ligand to a receptor protein, it is imperative to have charged counterbalancing ions located in the receptor. Hence, how can ligand binding (which requires charged counter ions) involve transmembrane amino acids when these are known to be lipophilic (i.e. non-polar, non-charged)? The answer appears to be that while transmembrane amino acids are lipophilic overall, side chain groups in critical amino acids are charged and thus provide the required counter ions [14].

When considering which regions in a receptor protein are important for ligand binding, it is important to remember that adrenoceptors are three-dimensional. Transmembrane domains in G protein-coupled receptors coalesce to form a binding pocket, permitting access from the extracellular space for binding of charged catecholamines [14, 42, 44]. In order to test which transmembrane regions are important in ligand binding, it is possible to take advantage of molecular biology techniques such as DNA "cutting and pasting" to form novel genes which encode synthetic receptors, specifically with alterations in the area of interest. For example, if regions of the β_2 adrenoceptor are gradually replaced with β_1 adrenoceptor starting from the amino terminus, ligand binding properties remain as would be expected for the β_2 adrenoceptor, until the third and fourth transmembrane regions are replaced with β_1 adrenoceptor [18]. At this point, agonist ligand binding properties change to those expected for the β_1 adrenoceptor even though the remainder of the receptor is still β_2 [18]. This suggests that the third and fourth transmembrane domains may be important in ligand binding [18, 42, 44]. Similar studies have determined that antagonist binding also involves the sixth and seventh transmembrane domains [23]. It is important to remember, however, that the seventh transmembrane may be close to the third and fourth transmembrane when the three-dimensional structure is taken into account. While synthetic chimeric receptors give an initial idea of which general regions are involved in ligand binding, it is important to know also which individual amino acids act as counter ions for ligands and are therefore critical in ligand binding. Mutation (or changing) of individual amino acids has also been performed using standard molecular biology techniques [31, 45], and distinct amino acids acting as counter ions for either agonists and antagonists have been identified for the β_2 adrenoceptor [42, 44]. These studies implicate distinct charged amino acids present in hydrophobic transmembrane areas as providing counter ions for the ligand cationic amine group, hydrogen bond interactions with catecholamine hydroxyl groups and aromatic interactions with the catecholamine ring.

G protein coupling

Transmembrane receptors interact with G proteins on or near the cytoplasmic surface of the cell

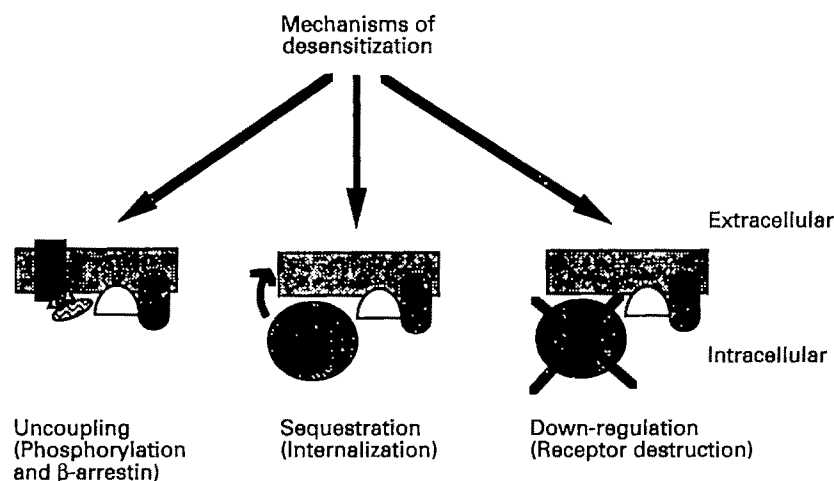


FIG. 8. Three basic mechanisms of desensitization have been discovered. The first involves receptor (black rectangle)-G protein (white half-circle) uncoupling. This includes receptor phosphorylation (triangles) and activation of other proteins such as beta arrestin (oval) which prevent receptor interaction with the G protein. The second mechanism is receptor internalization, or sequestration, and involves mobilization of the receptor to intracellular vesicles. Sequestered receptor can be recycled back to the surface once agonist stimulation terminates. The third mechanism of desensitization is receptor down-regulation, a process where sequestered receptors are destroyed (see text for details).

membrane. Hence it would be expected that the region(s) involved in G protein coupling would be part of the receptor cytoplasmic surface. Cytoplasmic regions of adrenoceptors in general include the three cytoplasmic loops and carboxyl terminus. Since the initial portion of the carboxyl terminus is anchored to the lipid membrane via palmitoylation at a critical cysteine residue, a fourth intracellular loop is actually formed. In general, regions known to be important in G protein coupling include the amino and carboxyl portions of the third intracellular loop, and the initial portion of the carboxyl terminus (fig. 6c). Part of the second intracellular loop may also be involved, but to a lesser extent. Detailed reviews of specific regions and residues important in receptor-G protein coupling are available elsewhere [18, 23, 33, 36, 43].

Regulation of adrenoceptors

Regulation of adrenoceptors becomes important when considering disease states involving the adrenergic nervous system, such as hypertension, congestive heart failure and acute tolerance to catecholamine drug therapy. One of the most common and intensively studied methods of regulating adrenoceptors is desensitization. Desensitization may be defined as a general biological phenomenon characterized by waning of a physiological response over time, despite the presence of a constant stimulus. It is important to remember that desensitization is therefore a functional definition describing a physiological end-point and does not imply in itself a specific mechanism. Desensitization may be thought of as an important response required to dampen extremes of physiology. For example, stimulation with adrenaline may increase heart rate significantly. Rapid heart rate is not well tolerated in the long term; desensitization therefore provides a protective mechanism to dampen heart rate response to adrenaline, gradually returning heart rate to normal.

Two general types of desensitization have been described, homologous and heterologous [36]. Homologous desensitization occurs when only the receptor which binds the stimulating agonist is desensitized (i.e. only beta adrenoceptors are desensitized to the beta adrenoceptor agonist isoprenaline and not other receptors). In contrast, heterologous desensitization occurs when stimulation of a distinct receptor results in the production of a second messenger which ultimately causes the desensitization of other receptor systems coupled to the same second messenger pathway (i.e. stimulation of the prostaglandin PGE_1 receptor which causes the production of cAMP, results in both PGE_1 receptor and beta adrenoceptor desensitization) [36].

While desensitization is a functional definition, three distinct mechanisms have been discovered to mediate this phenomenon (fig. 8). The first involves uncoupling of the receptor from the intermediary G protein. Receptor-G protein uncoupling occurs rapidly (within minutes to hours) following agonist exposure. Receptor phosphorylation is thought to result in inhibition of effective receptor-G protein interactions. Using the beta₂ adrenoceptor as a model, at small agonist concentrations (nmol litre⁻¹ range), cAMP-dependent protein kinase (protein kinase A) phosphorylates a site in the initial carboxyl terminus and also in the third intracellular loop [20]. At large agonist concentrations (i.e. $\mu\text{mol litre}^{-1}$), phosphorylation occurs via protein kinase A but also by another kinase called beta adrenoceptor kinase which phosphorylates agonist occupied beta₂ adrenoceptors on serine and threonine residues in the carboxyl terminus [2, 3, 20]. Phosphorylation via beta adrenoceptor kinase causes another protein molecule, beta arrestin [28] to bind to the receptor and effectively inhibit receptor-G protein binding [20]. Over a decade of intense research has led to detailed understanding of receptor sites of phosphorylation, enzymes involved and timing of events during receptor uncoupling in desensitization.

The reader is referred to several reviews of this topic [2, 3, 7, 20, 28, 40, 41]. However, the important point is that receptor-G protein uncoupling occurs rapidly without changes in cell surface receptor number.

A second mechanism of desensitization is sequestration. Sequestration refers to internalization of adrenoceptors during chronic agonist stimulation, a process which occurs in minutes to hours. It is important to note that sequestered receptor may be recycled back to the cell surface once agonist stimulation is discontinued. In spite of intensive study over the past decade, mechanisms responsible for sequestration have yet to be elucidated.

The third mechanism of desensitization is down-regulation, a slower process which occurs over hours to days. Like sequestration, down-regulation involves internalization of adrenoceptors. However, a further trigger is initiated in down-regulation that results in destruction of receptor protein, hence receptors cannot be recycled to the cell surface once agonist stimulation discontinues. Instead, new receptor protein must be made in order to increase adrenoceptor number back to baseline. In *in vitro* assays, cells exposed to agonist stimulation decrease the surface receptor number significantly after approximately 3 h. Long-term down-regulation may occur via increased receptor destruction or decreased receptor synthesis. Each of these potential mechanisms is under intense study and appears to be triggered by receptor phosphorylation [10]. Since desensitization is important in human disease and acute drug therapy, it is important to determine the mechanisms involved; this may one day enable specific inhibitors to be used to prevent desensitization when acutely required clinically.

CONCLUSIONS

Adrenergic receptors are important receptors in clinical medicine. Because of their transmembrane location, agonist occupancy is signaled via coupling to intermediary G proteins and ultimately activation of distinct second messenger systems. Because of intense study, adrenoceptors provide good models for G protein-coupled receptors in general. Recently, molecular biology techniques have enabled the discovery of genes encoding nine distinct adrenoceptor subtypes (three α_1 , three α_2 and three beta adrenoceptors), and have enabled detailed elucidation of overall structural features, as well as regions involved in ligand binding, G protein coupling and desensitization. The availability of genes encoding individual adrenoceptor subtypes will hopefully result in the elucidation of more subtype-selective drugs for use clinically with fewer side effects in the near future.

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SIGNAL TRANSDUCTION: G PROTEINS AND SECOND MESSENGERS

D. G. LAMBERT

The mechanisms underlying receptor–effector coupling are fundamental to our understanding of cellular function. A central issue in this area is how cell surface receptors transduce and amplify incoming signals. Other articles in this issue of the Journal cover the details of Ca^{2+} channel structure and their interactions with anaesthetic agents. This review concentrates on two of the most well characterized second messenger systems, adenylyl cyclase–cAMP and inositol polyphosphate– $[\text{Ca}^{2+}]_i$.

WHAT DO WE MEAN BY SIGNAL TRANSDUCTION AND WHAT IS A SECOND MESSENGER?

Signal transduction is the process by which a cell can convert an input into an appropriate output [51], for example conversion of muscarinic stimulation of glandular tissue into a secretory response. There are four elements between input and output in this basic system (see fig. 1). The agonist or first messenger (which may be a neurotransmitter or hormone) interacts with a specific cell surface receptor. The conformational change occurring in the receptor protein as a consequence of agonist binding enables the receptor to interact with the second element in the system, the G protein. The G protein transduces the signal to an amplifying enzyme (third component) whose activity elaborates a second messenger, the final component of the system. The second messenger will activate a cascade of enzymes which ultimately lead to an increase in protein phosphorylation and an output or response.

G PROTEIN-COUPLED RECEPTORS AND THEIR SECOND MESSENGER SYSTEMS

A basic understanding of the structure of G protein-coupled receptors is important to appreciate both the consequences of agonist binding and the sites at which G proteins interact. G protein-coupled receptors appear to have a common structure in that, of the receptors cloned to date, all span the plasma membrane seven times (which is why they have often

been named the magnificent seven) with three extracellular loops and three intracellular loops; the carboxy terminus of the protein is intracellular and the N terminus is extracellular. The remainder of this review will consider the muscarinic receptor family (fig. 2) [27, 59].

The muscarinic receptors are a family of five subtypes M1–M5; all have different amino acid sequences, but there is a great deal of similarity (homology) between certain of the subtypes in precise areas of the protein. For example, the third intracellular loop of the proteins show greatest homology between M1, M3 and M5 and between M2 and M4, but not between these groups. Also, M1, M3 and M5 subtypes couple to inositol polyphosphate generation and M2 and M4 subtypes inhibit the generation of cAMP. These findings point to the third intracellular loop of the protein being the site at which subtype selective messenger linkage occurs [34, 59] (i.e. the site of receptor G protein interaction). In an elegant series of experiments where chimeric muscarinic receptors have been created, it has been shown that if an M2 receptor is constructed with an M3 third intracellular loop, this M2 receptor (with a normal pharmacological profile) couples to the wrong second messenger system, inositol polyphosphate generation [68, 69]. There has also been suggestion, but no definite consensus, that the C terminal region may also be involved in the interaction with G protein [68]. The site of agonist interaction appears to involve spanning domains 3, 6 and 7, and if the receptor is redrawn in the helical wheel configuration, as shown in figure 2 (“birds eye view” of the extracellular surface of the membrane), agonist interaction may be seen clearly.

To date, there have been at least 100 G protein-coupled receptors identified (many have been cloned and sequenced) and for many the pharmacology or function has not been established [67]. Indeed the field is advancing so rapidly that in the period between writing this article and it going to press, many more receptor(s) will have come under the “cloners” scrutiny. Table I shows some of these G protein-coupled receptors and the second messenger system to which they couple.

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KEY WORDS

Mechanism of action: signal transduction. Receptors: G proteins, second messengers.

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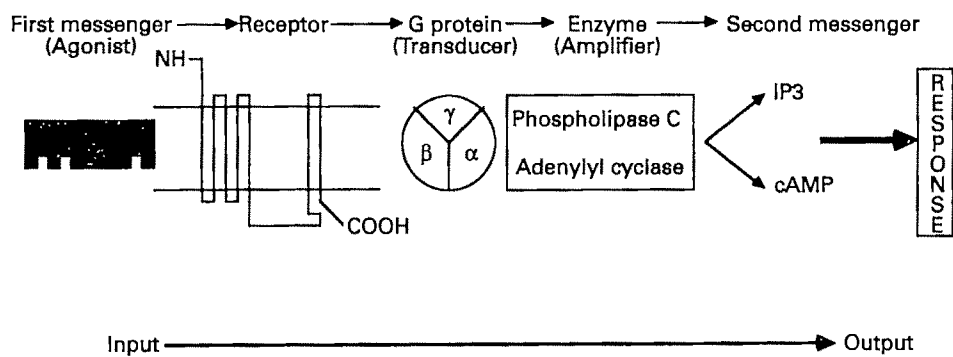


FIG. 1. Diagrammatic representation of the process of signal transduction; the conversion of receptor input into an appropriate output. The system consists of four basic elements: the receptor, G protein, amplifying enzyme and the second messenger. In this example, adenylyl cyclase-cAMP and phospholipase C-Ins(1,4,5)P₃ are shown.

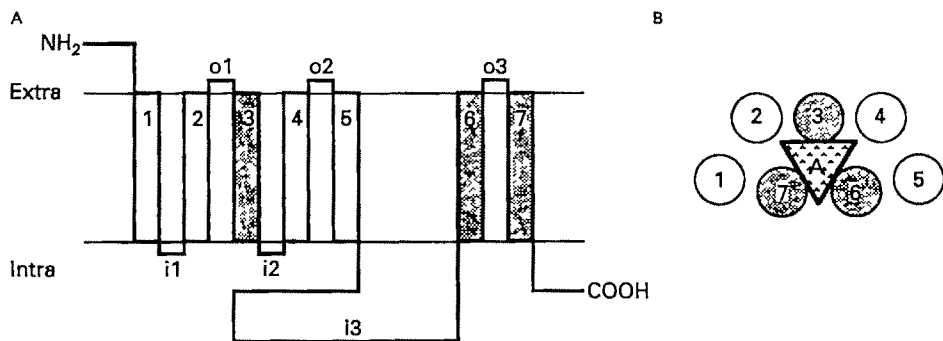


FIG. 2. Muscarinic receptor structure. A: The muscarinic receptor is a member of the seven transmembrane (TM1-7) spanning G protein-coupled receptors. The receptor has three intracellular (i1-i3) and three extracellular (o1-o3) loops. i3 = Site of G protein (alpha subunit) coupling. B: Helical wheel transformation of the receptor shows transmembrane domains 3, 6 and 7 forming the agonist (A) binding pocket. (See text for further description and [27]. Modified from [34].)

TABLE I. Examples of G protein-coupled receptors and the intracellular second messengers they generate. cAMP+ = Receptor stimulates generation of cAMP; cAMP- = receptor inhibits generation of cAMP; IP3+ = receptor stimulates generation of inositol(1,4,5)triphosphate (IP3) (see text and [67])

Receptor	Second messenger
Adrenoceptors	
Alpha _{1A/1B/1C}	IP3 +
Alpha _{2A/2B/2C}	cAMP -
Beta _{1/2/3}	cAMP +
Bradykinin (B ₁₋₂)	IP3 +
Calcitonin gene related peptide	cAMP +
Dopamine (D ₁₋₅)	IP3 + / cAMP -
Glutamate (metabotropic)	IP3 +
Histamine	cAMP + / IP3 +
5-HT (1A-1D/2/3/4)	cAMP - / cAMP + / IP3 +
Muscarinic (M1-5)	IP3 + / cAMP -
Opioid (μ/δ/κ)	cAMP -
Vasopressin (V _{1A/1B/2})	IP3 + / cAMP +

GUANINE NUCLEOTIDE BINDING (G) PROTEINS

Guanine nucleotide binding (G) proteins are a group of membrane associated proteins that transduce the agonist-receptor interaction into an intracellular response via interaction with a specific effector enzyme (fig. 1) [8, 9, 44, 49, 64]. As the name suggests, these proteins bind the guanine nucleotides GDP and GTP. In the inactive state, GDP is bound and when activated GDP is exchanged for GTP. G proteins are heterotrimers (i.e. they are composed of three structurally distinct subunits, alpha, beta and gamma). The beta and gamma subunits show good

homology across different G proteins. G proteins are best distinguished by differences in their alpha subunits (table II). Beta and gamma subunits are probably involved in anchoring the protein in the plasma membrane, whereas the alpha subunit has a number of functions to perform. It must interact with the receptor (see fig. 2), it must interact with the effector enzyme (e.g. adenylyl cyclase) and it must possess GTPase activity to enable inactivation. Next we consider the sequence of events between agonist-receptor interaction and activation of the effector enzyme, the G protein cycle (fig. 3) [64]. Agonist interaction with the receptor causes a conformational change in the receptor protein to enable interaction with the G protein. Bound GDP (inactive) is exchanged for intracellular GTP (fig. 3A). The alpha subunit dissociates from the beta-gamma complex (fig. 3B). Alpha-GTP may then interact with the effector enzyme (fig. 3C). The alpha subunit displays GTPase activity which converts bound GTP to GDP (fig. 3D) and inactivates the G protein. Finally, the alpha and beta-gamma subunits reassociate and the cycle begins anew. The cycle can be modified pharmacologically using non-hydrolysable analogues of GTP (GTP[S] and Gpp(NH)p); these cannot be broken down by the GTPase intrinsic to the alpha subunit and thus provide prolonged stimulation of the effector enzyme. The drawback with these analogues is that they are membrane impermeant and may only be used in membranes or permeabilized cells [70]. The activity of two bacterial toxins has simplified

TABLE II. Examples of different G proteins, their effector enzymes and tissue specific responses. AC = adenylyl cyclase, PLC = phospholipase C, $\text{Ins}(1,4,5)\text{P}_3$ = inositol (1,4,5) triphosphate, DAG = diacylglycerol, PDE = phosphodiesterase (see [44, 49, 64])

Agonist	G protein	Effector/Messenger	Tissue/Response
Glucagon	G_s	AC/Increased cAMP	Liver/Release of glucose
Opiates	G_i	AC/Reduced cAMP	CNS/Decreased Ca^{2+} entry
	G_o	$\text{K}^+/\text{Ca}^{2+}$ channels	Hyperpolarization/Decreased neurotransmission
Acetylcholine (M1/3/5)	G_q	PLC/ $\text{Ins}(1,4,5)\text{P}_3$	Various/Increased secretion
Light	G_t (transducin)	DAG/ Ca^{2+} entry cGMP-PDE	Neurotransmission Eye/Perception of light

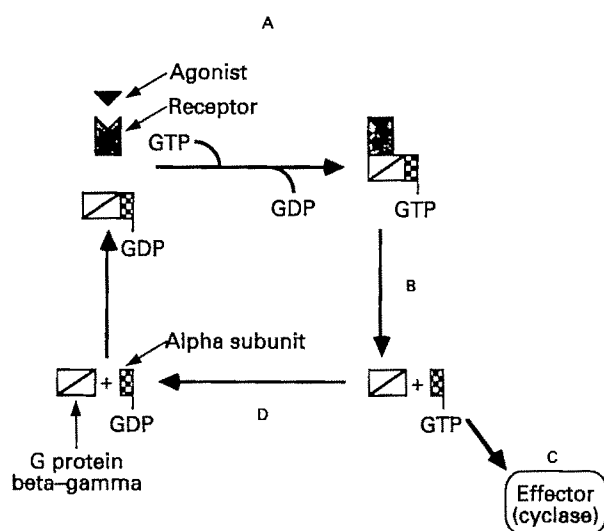


FIG. 3. Schematic representation of the G protein cycle. From top left, clockwise: agonist binding to its receptor promotes GDP for GTP exchange (A); G protein dissociation (B) allows the alpha-GTP subunit to activate the effector enzyme (C); an intrinsic GTPase converts GTP back to GDP (D) and the cycle is complete.

the classification of G proteins and has given clues to the mechanisms of disease. Pertussis toxin interacts with a class of G_i -like G proteins (e.g. G_i and G_o). Cholera toxin interacts with a different class of G proteins (e.g. G_s). In the case of pertussis toxin, this toxin ADP-ribosylates G_i (like) G proteins and prevents their interaction with the effector enzyme. Cholera toxin causes prolonged interaction of the G protein with the effector enzyme. For example, in diarrhoea associated with cholera, overstimulation of cAMP formation in the gut leads to oversecretion of water and electrolytes [64]. Recently, G proteins have been more accurately identified and classified using G protein specific antibodies [49].

ADENYLYL CYCLASE-cAMP

Adenylyl cyclase is the membrane bound enzyme that converts intracellular ATP to the second messenger cAMP [41–43]. Increased concentrations of cAMP activate a specific protein kinase enzyme (protein kinase A) and cause the phosphorylation events that lead to cellular responsiveness. Examples of cAMP coupled receptors are shown in table I. The activity of adenylyl cyclase can be regulated by receptor input in two ways (fig. 4). First, stimulatory receptors (R_s), for example CGRP and beta adren-

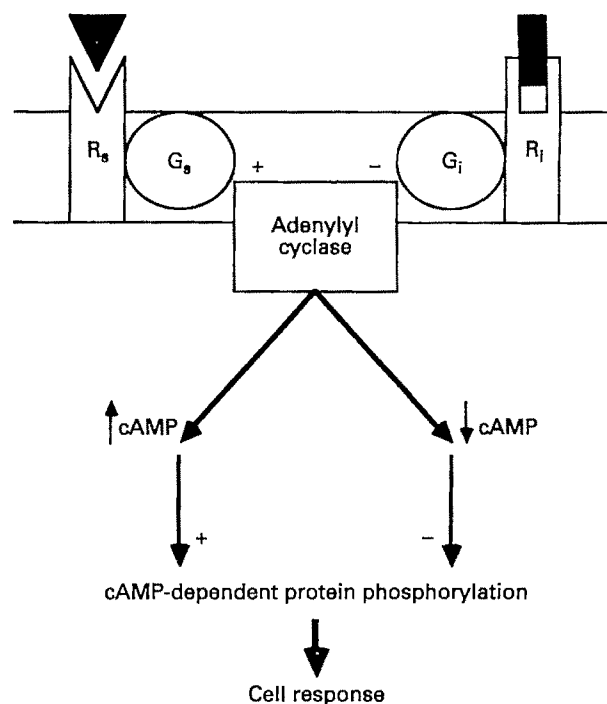


FIG. 4. Receptor regulation of adenylyl cyclase and cAMP formation. Membrane bound adenylyl cyclase is activated to increase cAMP via stimulatory receptors (R_s) acting through G_s (e.g. CGRP) or inhibited via inhibitory receptors (R_i) through G_i (e.g. opioids). Altered concentrations of cAMP alter protein phosphorylation and cellular responsiveness.

ergic receptors, couple via the stimulatory G protein (G_s) to activate the enzyme and increase the intracellular concentration of cAMP. Inhibitory receptors (R_i), for example opioid and α_2 adren-ergic receptors, couple via the inhibitory G protein (G_i) and reduce cAMP formation. The biological signal produced by cAMP is terminated by cellular phosphodiesterase activity converting cAMP to 5'-AMP. There are several ways in which we may interfere with the system pharmacologically. The intracellular concentration of cAMP can be increased artificially using the adenylyl cyclase activator forskolin (fig. 5) and cAMP breakdown may be inhibited by phosphodiesterase inhibition (e.g. using the methylxanthine, IBMX) [41–43]. Indeed, in a system where cAMP breakdown is inhibited and adenylyl cyclase is activated with forskolin, many groups have examined the G_i linked inhibition of cAMP formation. An example of such a study is shown in table III, comparing opioid and α_2 receptor mediated cAMP inhibition [38]. As men-

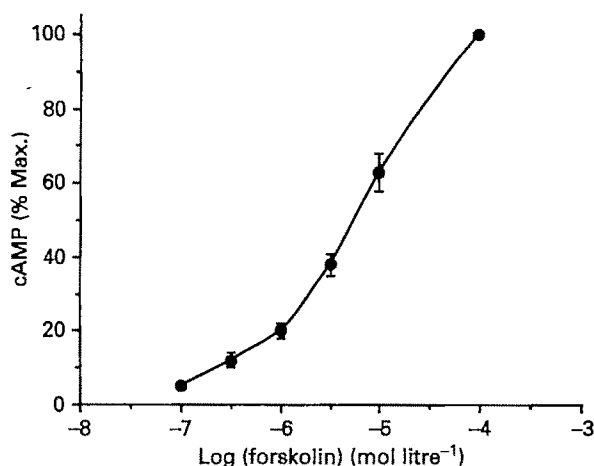


FIG. 5. Effect of forskolin on cAMP formation in SH-SY5Y human neuroblastoma cells (mean $\pm$ SEM, $n = 5$). Cells were incubated in the presence of the phosphodiesterase inhibitor IBMX and forskolin. Forskolin directly activates adenylyl cyclase and increases cAMP. In this system G_i linked receptors can be studied by the degree to which they inhibit forskolin stimulated cAMP formation (see table III).

TABLE III. Effect of adrenergic and opioid agonists on forskolin ($10 \mu\text{mol litre}^{-1}$) stimulated cAMP formation (mean (SEM)) in undifferentiated SH-SY5Y human neuroblastoma cells. (Modified from Lambert and Nahorski [38] and unpublished observations)

Agent	Concentration	% Inhibition of forskolin stimulated cAMP formation
Adrenaline	$1 \mu\text{mol litre}^{-1}$	61.3 (13.8)
+ pertussis toxin	100 ng ml^{-1}	4.6 (0.4)
Fentanyl	$1 \mu\text{mol litre}^{-1}$	49.8 (5.6)
Morphine	$1 \mu\text{mol litre}^{-1}$	26.2 (3.5)

tioned above, cAMP can be increased by the action of cholera toxin on G_s and the inhibitory action of G_i linked receptors may be prevented by pertussis toxin (table III) [64].

INOSITOL POLYPHOSPHATE- $[\text{Ca}^{2+}]_i$

Activation of a wide variety of cell surface receptors leads to the breakdown of a specific membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP₂) to yield two biologically active second messengers, inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃) and diacylglycerol (DAG) [5-7, 14, 30, 40]. The breakdown of PIP₂ is achieved by a membrane associated enzyme phospholipase C. In common with the cAMP signalling system, the receptor is coupled to the effector (phospholipase C) by a transducing G protein. This G protein is, in many cases, the recently identified G_q [62] (fig. 6). There is now irrefutable evidence that Ins(1,4,5)P₃ releases Ca^{2+} from an intracellular store [30, 50, 52]

to PIP₂ hydrolysis is often complex [36, 37, 40]. For example, in SH-SY5Y human neuroblastoma cells, stimulation of M3 muscarinic receptors leads to a biphasic increase in intracellular Ca^{2+} (fig. 7) [36]. The peak phase is a result of release of Ca^{2+} from an intracellular Ins(1,4,5)P₃ sensitive store and the plateau phase is caused by Ca^{2+} entry across the plasma membrane [36, 39].

Measurements of Ins(1,4,5)P₃ mass concentration (fig. 7), show close correlation with the Ca^{2+} signal, indicating the involvement of this polyphosphate in the release of stored Ca^{2+} [35, 36]. Indeed, in permeabilized SH-SY5Y cells, exogenous Ins(1,4,5)P₃ has been shown to release stored Ca^{2+} [71]. It appears that the long lasting increase in Ins(1,4,5)P₃ (fig. 7), is the result of Ca^{2+} activation of phospholipase C [60], the source of this Ca^{2+} being via influx [40] (see below). Intracellular Ins(1,4,5)P₃ produced by receptor stimulation is handled by the cell in two different ways. First, it may be further phosphorylated by a 3-kinase enzyme to produce inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P₄) and a variety of roles have been ascribed to this polyphosphate [30]. The biological signals produced by both of these second messengers are terminated by a 5-phosphatase enzyme to yield the inactive Ins(1,3,4)P₃ and Ins(1,4)P₂ from Ins(1,3,4,5)P₄ and Ins(1,4,5)P₃, respectively. These polyphosphate intermediates are subsequently dephosphorylated to inositol which can then be re-incorporated into the membrane pool of phosphoinositides and thus completes the cycle [60].

It is assumed that overactivity of the polyphosphoinositide cycle occurs in manic depressive illness and that the anti-manic drug, Li^+ , blocks inositol monophosphatase, thereby reducing the membrane pool of phosphoinositides and interrupting the cycle [53]. The cycle can be regulated in several subtle ways, the most notable being the Ca^{2+} sensitivity of the enzyme phospholipase C [60], in that Ca^{2+} released from intracellular stores or gated from the extracellular space may activate phospholipase C and cause further generation of Ins(1,4,5)P₃ and DAG. DAG is a lipid soluble second messenger molecule which remains in the plane of the plasma membrane to activate another membrane bound enzyme, protein kinase C, and once again this enzyme causes protein phosphorylation and cellular responsiveness. It is noteworthy that phosphoinositides have also been found in the nuclear membrane [15]. Their function is unclear but they could be involved in intranuclear Ca^{2+} homeostasis. Therefore, by occupation of a receptor linked to phospholipase C, it is evident that a variety of second messengers may be generated and, depending on the intracellular targets present, a diverse array of cell

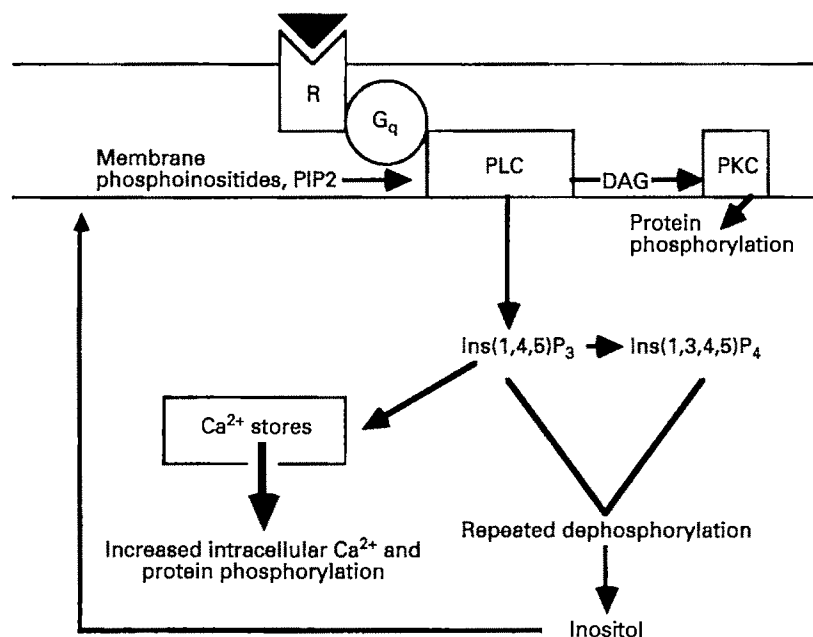


FIG. 6. Receptor regulation of phospholipase C activity and Ins(1,4,5)P₃ generation. Membrane bound phospholipase C is activated by G_q linked receptors (e.g. muscarinic M1, M3, M5) to increase PIP₂ hydrolysis and yield two biologically active second messengers; water soluble Ins(1,4,5)P₃ and lipid soluble diacylglycerol (DAG). The latter activates protein kinase C (PKC) and the former releases Ca²⁺ from an intracellular store. Ins(1,4,5)P₃ produced by receptor stimulation is either phosphorylated to Ins(1,3,4,5)P₄ (which may have some biological activity) or dephosphorylated to inositol. Increased Ca²⁺ and protein kinase C activity cause increased protein phosphorylation and alter cellular responsiveness.

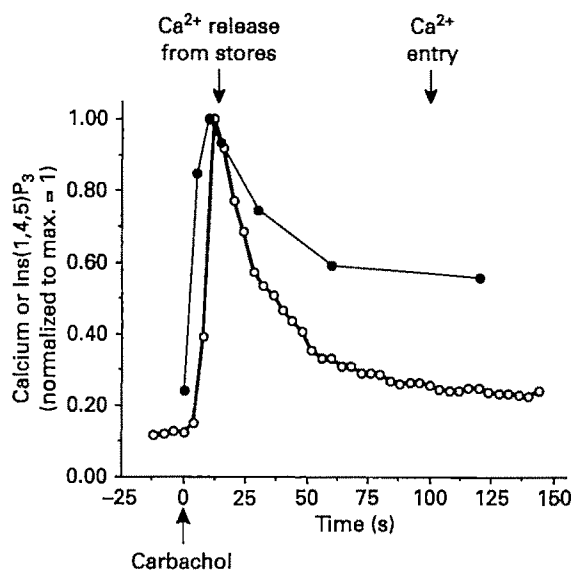


FIG. 7. Carbachol (1 mmol litre⁻¹) stimulates Ins(1,4,5)P₃ production and increases intracellular Ca²⁺ in SH-SY5Y human neuroblastoma cells. The Ca²⁺ signal (measured using the Ca²⁺ sensitive dye fura-2) to carbachol (○) is biphasic with the peak a result of Ca²⁺ release from intracellular stores and the plateau phase a result of Ca²⁺ entry. The Ins(1,4,5)P₃ (●) time course is also shown. Ins(1,4,5)P₃ peaks before Ca²⁺, indicating the former mediates release of the latter. The increased concentrations of Ins(1,4,5)P₃ at 2 min result from Ca²⁺ entry stimulating phospholipase C (data taken from [35, 36]).

systems possible, for example Ca²⁺ release from intracellular stores (mediated by Ins(1,4,5)P₃) or gated from the exterior could potentially activate adenylyl cyclase allowing intracellular Ca²⁺, Ins(1,4,5)P₃ and cAMP to be regulated by a single class of receptor.

It is also worth noting that other second messenger systems do exist, for example guanylate cyclase-cGMP, but space does not permit a full description and the reader is referred to the following articles [25, 32].

AMPLIFICATION

From the preceding sections it is clear that with a multistep signal transduction process, scope exists for amplification of an incoming signal. Consider the possibility that one molecule of glucagon binding to its receptor may interact with 10 molecules of G_s and each molecule of G_s may interact with 10 molecules of adenylyl cyclase to produce 10 molecules of cAMP: the initial signal has been amplified 10³ fold. Hence a sensitive response can be mounted to small levels of stimulation. It is not hard to imagine that a large input could be catastrophic in that intracellular messengers could build up to concentrations that would "swamp" the cell. Fortunately, amplification is rarely so large and reduction steps (using the above example, not every G protein will find a molecule of adenylyl cyclase to activate) must occur.

INTERACTION OF ANAESTHETIC AGENTS WITH SIGNAL TRANSDUCTION

In agreement with the Meyer-Overton "rule" that anaesthetic potency correlates with lipid solubility [48, 56, 63], Franks and Lieb suggested that the site of action of anaesthetic agents is likely to be a cellular lipid or a cellular component surrounded by lipid. The most obvious target appears to be the plasma membrane where anaesthetics could potentially: (a) increase membrane fluidity, (b) increase membrane phase transition, (c) cause changes in the dimensions

TABLE IV. Examples of the effects of anaesthetic agents on receptor binding. AR = Adrenoceptor, MR = muscarinic receptors. Bmax = Maximum number (density) of binding sites

Anaesthetic	Tissue	Receptor	Effect	Ref.
Halothane (1.25 %)	Rat brain slices	Alpha _{1/2} -AR	No effect	[2]
Halothane (3-5 %)	Canine myocardium	Beta-AR, MR	No effect	[3]
Halothane (2 %) and nitrous oxide (100 %)	Guineapig brain	μ and κ opioid	Decrease μ + κ affinity and κ Bmax	[55]
Halothane (to 18 mmol litre ⁻¹) Ketamine (to 2 mmol litre ⁻¹) Ethanol (to 2000 mmol litre ⁻¹)	Rat cortical slices	Beta-AR	No effect	[54]
Halothane (0.5-5 %)	Rat brain membranes	MR	No effect on Bmax. Increased affinity	[1]

of the membrane, (d) alter membrane permeability or (e) interact with proteins [20, 21]. The latter possibility appears the most attractive but are anaesthetic agents capable of interacting with proteins directly or indirectly via action at the lipid-protein interface? It is important to remember that the cell has more than one lipid bilayer and the cell membrane may not be the only site of action. We must also look to the nucleus, mitochondria, endoplasmic reticulum, secretory granules, etc, all of which are surrounded by membranes, as possible targets.

There are various steps in the signal transduction pathway as possible sites of action (many of which are in the lipid environment of the plasma membrane) of anaesthetic (particularly volatile) agents. Membrane ion channels have been deliberately excluded as they will be covered by other reviews in this issue. It is possible to identify four target groups (see figs 1, 8): (a) binding of agonist to receptor, (b) G protein function, (c) effector activity (adenylyl cyclase, phospholipase C) and (d) other intracellular sites (Ca²⁺ stores, cellular kinases).

There have been several studies detailing the effects of anaesthetic agents on ligand-receptor binding. However, all have produced variable and what appear to be tissue-specific differences. The most widely studied systems are adrenergic, muscarinic and opioid, and the results of these are summarized in table IV [1-3, 54, 55].

It is worth defining some of the terms used with reference to receptor binding. Bmax (receptor density) and the affinity of a drug (its K_d) may be determined directly where a radiolabelled ligand is available. This is accomplished by adding increasing concentrations in the presence of a fixed number of receptors until the specific receptor binding saturates (Bmax): K_d is the concentration of ligand that produces half maximum binding [58]. The K_d of a ligand is like a signature for that particular receptor and if an anaesthetic agent were to alter the K_d of a ligand for its receptor, it is likely to be interfering with ligand-receptor binding. It is also possible that anaesthetic agents could alter Bmax, by either increasing (perhaps by up-regulation) or decreasing (perhaps by producing desensitization) receptor density. Drug affinity may also be calculated using a

fixed concentration of radiolabelled ligand and increasing concentrations of unlabelled drug, which displace the label. The concentration producing half maximum displacement is related to affinity [12]. Halothane at clinical concentrations has no effect on adrenoceptor (alpha or beta) binding to either brain or cardiac tissue [2, 3], yet this volatile agent has variable effects on muscarinic receptor affinity (table IV) [1, 2]. Opioid receptors are of obvious clinical importance as opioids are commonly given in combination with inhalation anaesthetics. Halothane (and nitrous oxide) appear to decrease both μ and κ receptor affinity and also κ receptor Bmax in guineapig brain [55]. The consequences of these effects are unclear but may be mutually antagonistic! Clearly this is an area in need of further investigation.

Another vital component in the signal transduction pathway that is intimately associated with the plasma membrane is the G protein. Studies on the effects of opioids on G_i/G_o are numerous, but in these examples the agonist "needs" the G protein to transduce its signal [13, 47]. Unfortunately, for volatile anaesthetics the effects are less well known and have been addressed by looking at modulation of the agonist-receptor-G protein interaction. As described above, the affinity of an unlabelled agonist may be determined by displacement of a labelled drug: if this experiment is performed in washed membranes (no GTP), then two populations of receptors exist, those pre-coupled to the G protein (high affinity for agonist) and free receptors (low affinity for agonists). If GTP is added (see fig. 3), G protein activation occurs and the high affinity binding moves to low affinity (i.e. G protein pre-coupled will dissociate) [64].

Thus in an experiment where there is a shift in the proportion of high affinity receptors to low affinity upon addition of GTP, it would be possible to examine any gross effects of volatile anaesthetics on G protein function. In two separate studies (both in rat brain), halothane was shown to either eliminate the high-low affinity shift or to have no effect [1, 2] upon carbachol binding to muscarinic receptors. Halothane appears to have no effect on alpha and beta adrenoceptor G protein interaction [2].

Okuda, Miyazaki and Kuriyama adopted a different approach to study G proteins. They

TABLE V. *Effects of halothane on cAMP formation*

Tissue	Halothane concn	Effect on cAMP concn	Ref.
Rat/cat myocardium	1-3 %	Decrease noradrenaline stimulated	[23]
Rat brain (cerebellum, brain stem, pituitary hypothalamus)	1 %	Increase <i>in vivo</i> concn	[31]
Rat whole brain	1.5-2 %	Increase <i>in vivo</i> concn	[46]
Rat cerebrocortical slices	0.9-18 mmol litre ⁻¹	Decrease noradrenaline stimulated	[54]
Rat liver homogenates	3-7 %	Increase basal and glucagon stimulated	[4]
Mouse heart	1.2 %	Decrease whole tissue content	[66]
Rat cerebrocortical slices	1.25 %	No effect on basal or noradrenaline stimulated	[2]

examined the binding of the non-hydrolysable analogue of GTP, [³H]Gpp(NH)p (this is not broken down by the intrinsic GTPase and produces long lasting stimulation of the effector enzyme). Halothane and ketamine had no effect on the binding of [³H]Gpp(NH)p, but at large concentrations, ethanol produced significant inhibition [54]. In a curious report, examining the interaction of halothane and morphine on the guineapig ileum, it was shown clearly that the relaxant effects of morphine were unaffected by pertussis toxin pretreatment, yet the relaxant effect of halothane was reduced markedly (half maximum inhibition shift from 2 % halothane to an extrapolated 9 % halothane) [57]. The mechanisms underlying this response are unclear, but pertussis toxin was administered to the whole animal. At the time of writing this review, the author is unaware of any reports of anaesthetic interaction with the intrinsic GTPase associated with the alpha subunit. Clearly inhibition would prolong the activation of the effector component of transduction.

The effects of halothane on cAMP formation have been reported by several authors using a variety of experimental paradigms. These vary from measurements of whole tissue contents after anaesthesia [31, 46, 66] to examining the effects on agonist-driven responses [2, 4, 23, 54] (table V). What is clear, and in accord with previous sections, is that no consistent effects have been observed. In neuronal tissues, halothane increases cAMP concentrations *in vivo* [31, 46], decreases noradrenaline stimulated cAMP formation [54] or is ineffective [2]. In cardiac tissue, a general decrease is observed [23, 66], whilst in the liver, halothane increases both basal and glucagon stimulated cAMP formation [4]. These experiments are attempting to mimic (or to examine directly) what would happen *in vivo*. They do not address the question, what is the effect of halothane on adenylyl cyclase? From the preceding section it is clear that halothane may interact with receptor binding and may interact with the G protein. Studies in a simple preparation where adenylyl cyclase may be activated directly, for example with forskolin, may shed some light as to the action of halothane (and other volatile agents). It may be that the tissue differences observed reflect differences in the dis-

tribution of Ca²⁺ sensitive and insensitive forms of adenylyl cyclase [17, 24, 33]. Therefore, rather than acting directly, halothane may act indirectly by increasing intracellular Ca²⁺ [28] and activating a Ca²⁺ sensitive adenylyl cyclase. This idea needs testing. Halothane has also been shown to decrease cerebellar cGMP concentrations and motor activity [65].

Three studies on the effect of halothane on inositol polyphosphate formation are of interest [2, 26, 61]. All use the Li⁺ block technique to measure total inositol (poly)phosphate formation. Membrane polyphosphoinositides are labelled with [³H]inositol and the tissue incubated in the presence of agonist and Li⁺. Li⁺ blocks the monophosphatase causing the build up of inositol monophosphate. Thus in the presence of Li⁺, agonist stimulation is measured as an increase in the concentration of [³H]monophosphate. The major drawback with this technique is that the biologically active second messengers, Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ are not measured and it would be difficult to detect transient changes in their concentrations [53]. Studies using the newly available mass assays are needed [10, 11].

Bazil and Minneman [2] were unable to demonstrate any effects of halothane (1.25 %) on basal, noradrenaline, carbachol, histamine or serotonin stimulated inositol phosphate formation in rat brain cortical slices using a Li⁺ block. In a recent study, Sill and colleagues showed that halothane interfered with vasopressin stimulated inositol phosphate formation and subsequent generation of an intracellular Ca²⁺ signal in A7r5 cultured vascular smooth muscle cells. Halothane, 1 % and 2 %, decreased vasopressin stimulated inositol phosphate formation by 46 % and 61 %, respectively. The intracellular Ca²⁺ response consisted of Ca²⁺ entry and Ca²⁺ release from intracellular (presumably Ins(1,4,5)P₃ sensitive) stores. Halothane reduced significantly both components. A decrease in Ca²⁺ released from intracellular stores is consistent with a reduction in the concentration of Ins(1,4,5)P₃, although this polyphosphate was not measured directly [61]. Also, using the Li⁺ block technique, Hassessian, Prat and Couture demonstrated recently that pentobarbitone caused significant inhibition of basal, substance P

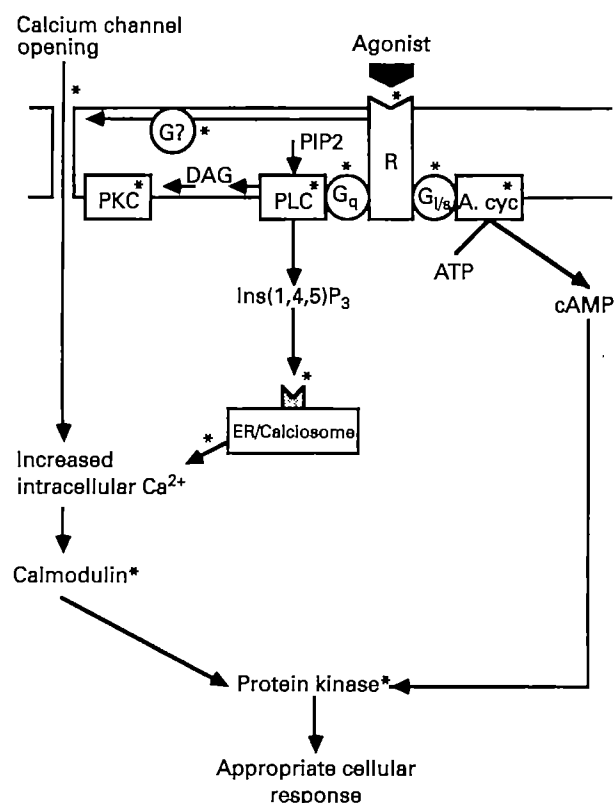


FIG. 8. Possible targets for the site of action of anaesthetic agents (see text). Anaesthetic agents could interact at all steps in the signal transduction pathway (*): agonist-receptor, receptor-G protein, G protein-effector coupling, second messenger generation or at some other intracellular site. R = receptor, G = G protein, PLC = phospholipase C, A.cyc = adenylyl cyclase; ER = endoplasmic reticulum Ca²⁺ store.

stimulated and carbachol stimulated inositol phosphate formation in spinal cord and cerebral cortex of the rat [26].

Some of the remaining possible targets must lie in the cytoplasm, cellular kinases that are activated by intracellular second messengers, mitochondria and possibly intracellular Ca²⁺ stores [16, 28]. There is evidence from the study of malignant hyperthermia to suggest an interaction of volatile anaesthetics with intracellular Ca²⁺ stores [45]. Certainly halothane has been shown to release Ca²⁺ from internal stores in normal tissue and to a greater extent in malignant hyperthermia [18, 19]. What is the nature of this interaction? The intracellular Ins(1,4,5)P₃ sensitive store is a membrane bound structure with an Ins(1,4,5)P₃ receptor, which probably acts as a Ca²⁺ channel, located on its surface [22, 52]. The previous discussion could equally relate to this structure with effects, for example on membrane fluidity, interaction with protein or the Ins(1,4,5)P₃ receptor.

Only with answers to the basic questions highlighted (fig. 8) in this and other reviews may we begin to unravel the complexities underlying the state of anaesthesia. The clues are there to be investigated further, and hopefully over the next few years an hypothesis to explain the mechanisms of anaesthesia will emerge.

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EFFECTS OF ANAESTHETICS ON UPTAKE, SYNTHESIS AND RELEASE OF TRANSMITTERS

R. GRIFFITHS AND R. I. NORMAN

While the molecular basis of the action of most anaesthetic agents is unknown, it is a commonly held view that general anaesthetic drugs have a more pronounced effect on synaptic mechanisms in the central nervous system (CNS) than on the propagation of electrical signals along axons [39]. A more contentious issue relates to whether or not anaesthetic agents act generally, producing a range of metabolic alterations which together result in anaesthesia or at, as yet, unidentified specific sites in neuronal mechanisms [25].

Sensory information is passed from cell to cell in the CNS by neurotransmitters. These transmitter substances are released in the synaptic cleft between adjacent cells and, depending on the particular synapse, can interact with presynaptic and/or postsynaptic receptors. The postsynaptic actions of transmitter substances are mediated classically via excitatory postsynaptic potentials (EPSP) or inhibitory postsynaptic potentials (IPSP) and the size of these can be modulated by the regulation of transmitter release from the nerve terminal by the interaction of neurotransmitter substances at receptors in the presynaptic membrane.

The diversity of different transmitter substances and the complex interplay between transmitter systems results in a large number of potential sites of action of anaesthetic agents (fig. 1).

EXPERIMENTAL SYSTEMS

Although there have been a number of *in vivo* studies [4, 9, 55–57, 65], by far the majority of studies on the action of anaesthetic agents on transmitter systems have been carried out in *in vitro* preparations. Three major experimental paradigms, namely synaptosomes, brain slices and cells in culture, have been used in *in vitro* studies (fig. 2). Each of these systems has limitations and caution must be exercised in extrapolating conclusions from these studies to the clinical situation.

Synaptosomes are pinched off nerve endings that are formed when brain tissue is homogenized. The synaptosome fraction is separated from particulate

material containing mitochondria and other cell debris by centrifugation in density gradients of either sucrose [27], Ficoll [11] or Percoll [22]. The resulting synaptosomes are sealed plasma membrane vesicles which contain the secretory machinery and mitochondria necessary for transmitter metabolism and are a good model for the study of the uptake and release of neurotransmitters (fig. 3). The synaptosome preparation consists of a mixed population of nerve endings containing many different transmitter types which permits the study of the metabolism of a number of neurotransmitters in a single system. The major limitations of the synaptosome preparation are that there is the potential for non-physiological cross-talk between transmitter systems and that the influence of supporting cells and other

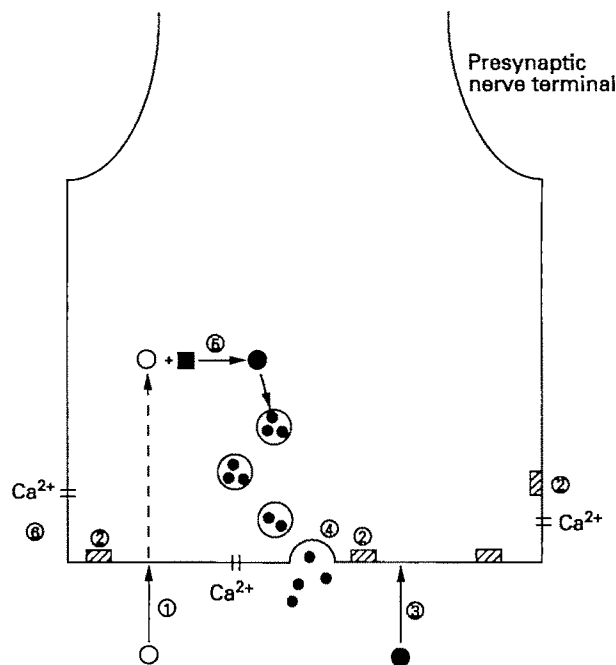


FIG. 1. Diagrammatic representation of a presynaptic nerve terminal showing potential sites of anaesthetic drug interaction. 1 = Precursor uptake, 2 = presynaptic receptors, 3 = released transmitter reuptake, 4 = transmitter release, 5 = transmitter synthesis, 6 = presynaptic calcium entry.

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KEY WORDS

Anaesthesia: mode of action. Neurotransmitters: release, synthesis, uptake.

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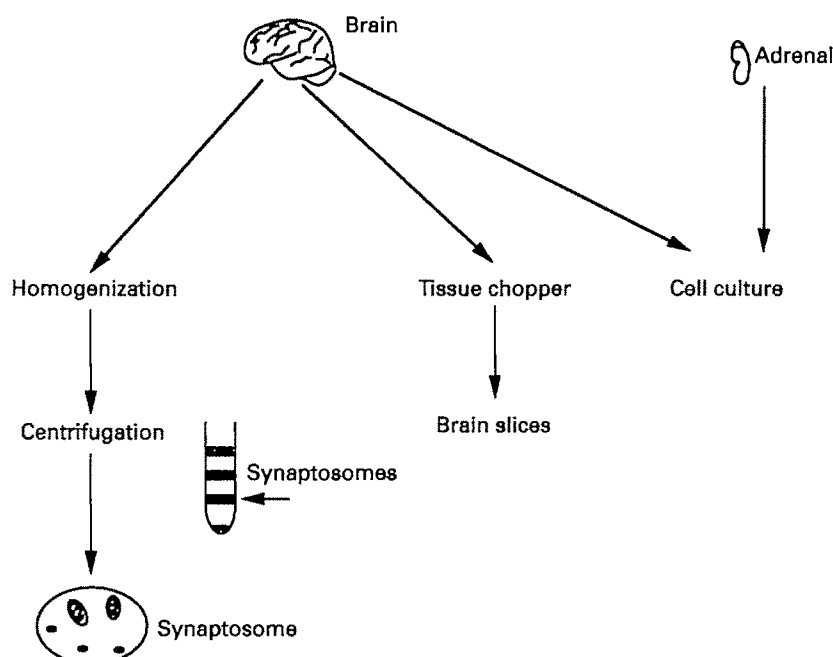


FIG. 2. Tissue preparations available to study neurotransmitter metabolism *in vitro*.



FIG. 3. Electron micrograph of a synaptosome prepared from rat cortex. m = Mitochondria, pm = plasma membrane, v = synaptic vesicle.

neurones has been removed by the process of shearing off the nerve endings. For a fuller consideration of the synaptosome preparation see McMahon and Nicholls [40].

Brain slice (pyramid) preparations have also been used extensively to study neurotransmitter metabolism. This is a more complex preparation, as some functional connections are maintained. Possibly the simplest *in vitro* preparations are neuronal and neuronal-like cells in culture, which permit the study of intact, isolated cell types. Again, the absence of functional tissue organization cannot be ignored and the relevance of observations made on non-

neuronal cells, such as chromaffin cells, and cells which may be only partially differentiated must be considered.

NEUROTRANSMITTER CLASSIFICATION

Neurotransmitter systems have been subdivided according to their classification into (1) amino acids, (2) other classical transmitters and (3) neuropeptide systems [40]. In this review we concentrate on the group 1 transmitters, including aspartate, glutamate, gamma-aminobutyrate (GABA) and glycine and the group 2 transmitters, including the catecholamines,

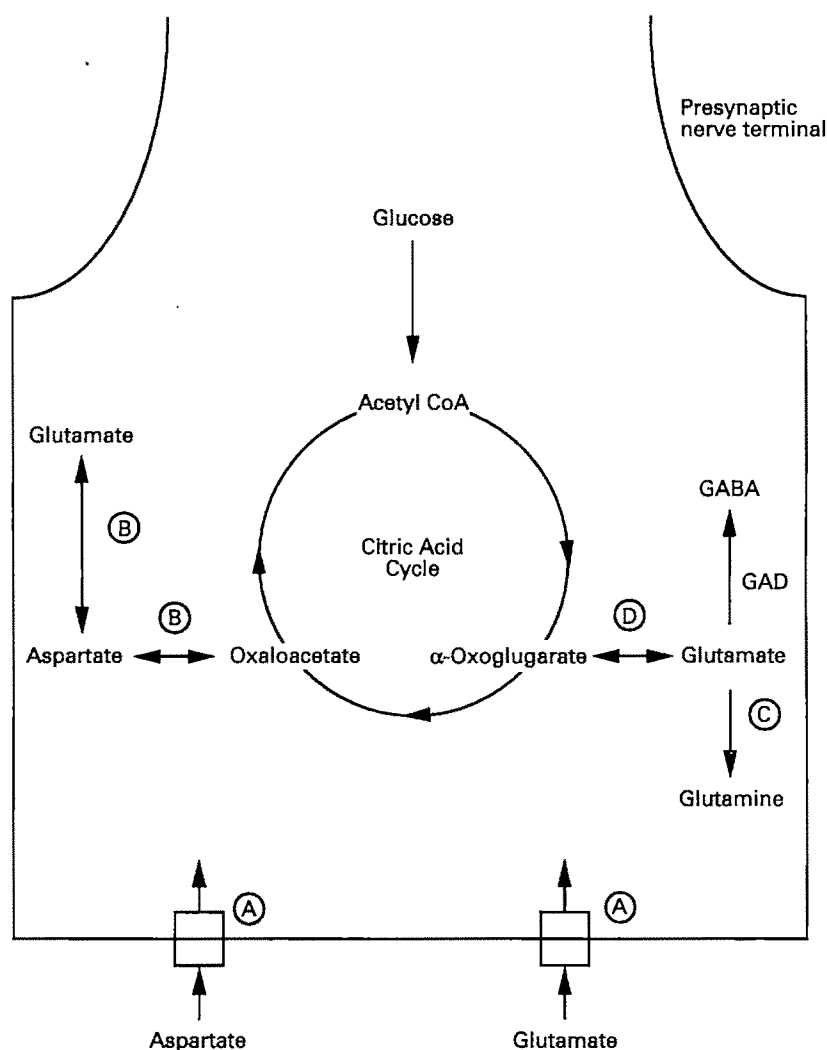


FIG. 4. Diagrammatic representation of a presynaptic nerve terminal showing the relationship between the metabolism of excitatory amino acid neurotransmitters and GABA. A = Aspartate glutamate co-transporter, B = aspartate aminotransferase, C = glutaminase, D = glutamic acid decarboxylase. GAD = Glutamic acid decarboxylase.

dopamine, noradrenaline and adrenaline, 5-hydroxytryptamine (5-HT) and acetylcholine, as little work has been directed to the effects of anaesthetic agents on group 3 transmitter systems.

GROUP 1—AMINO ACID TRANSMITTERS

Excitatory transmitters: glutamate and aspartate

Glutamate and aspartate are both believed to act as excitatory neurotransmitters in the mammalian brain [24]. In particular, neurotransmission mediated by glutamate has been implicated in learning and memory, neurodegenerative disorders, epilepsy and the effects of hypoxia, ischaemia and possibly other neurological and psychiatric states [16, 20].

Biosynthesis of glutamate can occur via two pathways, either from the deamination of glutamine or from the tricarboxylic acid cycle [33] (fig. 4). It would appear that most of the glutamate released following depolarizing stimuli is derived originally from glutamine [33]. After release into the synapse, signal transmission by the excitatory amino acids is mediated at the postsynaptic membrane by receptors for these neurotransmitters. These are classified into

three classes, N-methyl-D-aspartate (NMDA), quisqualate and kainate, depending on their pharmacology [69]. The NMDA receptor subclass is of particular importance to the anaesthetist as ketamine is a non-competitive antagonist at this receptor [72]. Postsynaptic events are terminated largely by the uptake of glutamate and aspartate into neighbouring glial cells. In view of their wide distribution and suggested roles in the CNS, the excitatory amino acid transmitter systems constitute an important potential target for anaesthetic action.

Release. In early studies of brain slices, barbiturates were shown to decrease the release of aspartate from electrically stimulated rat lateral olfactory tract slices in a dose-dependent manner [19]. Similarly, depression of potassium-stimulated aspartate release has been observed also in rat brain slices in the presence of pentobarbitone, methohexitone and thiopentone [5, 52, 68]. However, a biphasic pattern of release was observed in a rat thalamic slice preparation in which small concentrations of thiopentone (10^{-6} mol litre $^{-1}$) and methohexitone (10^{-6} mol litre $^{-1}$) enhanced release and greater doses decreased release of D-aspartate and GABA [37].

The greater concentration of thiopentone was within the clinical range but that of methohexitone was greater than clinical concentrations. The mechanism of the biphasic response in excitatory amino acid release in response to barbiturates remains unresolved, but may involve indirect effects on GABA transmission [37]. Depolarization-induced calcium uptake in the nerve terminal, which triggers transmitter release, has been shown to be inhibited by thiopentone and pentobarbitone [10]. Hence, alteration in calcium handling by the nerve terminal may provide an explanation for the action of barbiturates on excitatory amino acid transmission.

The steroid anaesthetic hydroxydione is the only other agent which has been shown to inhibit potassium-stimulated aspartate efflux [52]. Other anaesthetic agents, including Althesin (a combination of alphaxalone and alphadolone), ketamine, urethane and halothane have been reported to have no effect or to enhance the release of aspartate from rat brain slice preparations [5, 37, 52]. At clinical doses, 1–2% halothane had no significant effect on the efflux of [^3H]aspartate from rat brain slices [5], although increased aspartate efflux has been observed at larger concentrations of halothane (4–8%) which are considerably greater than the clinical dose range. In contrast, a recent study investigating glutamate release from mice synaptosomes [32] demonstrated an increase in release when the preparation was exposed to clinical concentrations of halothane and enflurane; the increase in release being greater for enflurane than for halothane at equivalent concentrations. Interestingly, the excitatory amino acids are implicated in the pathogenesis of some forms of epilepsy [20]: it is possible that the enhanced release of glutamate in the presence of enflurane could contribute to the electroencephalographic abnormalities seen with this agent [67].

In vivo measurements of excitatory amino acid release, by monitoring brain concentrations of ascorbate voltammetrically, have shown that anaesthesia with chloral hydrate reduces the release of excitatory neurotransmitters and that transmitter release recovers as the animal recovers [56]. These observations provide *in vivo* evidence for the interruption of excitatory neurotransmitter release by an anaesthetic agent during the process of anaesthesia.

From the limited evidence available, it is possible that some anaesthetics may act on synaptic transmission mediated by the excitatory amino acids [19, 32, 42, 52, 56, 60]. Perhaps as important, other agents would appear not to be implicated [5, 52]. Together, these results suggest a possible selectivity of action of some anaesthetic agents for the excitatory amino acid release mechanism. The absence of a "general" effect on these transmitter mechanisms is also observed for the volatile agents, as while halothane, and particularly enflurane, increased transmitter release, diethyl ether had no effect in the same study [32], suggesting selectivity of action of anaesthetic agents on the excitatory transmitter systems.

Recently, a new compound riluzole (54274 RP), has been found to have possible anaesthetic actions

and pharmacologically this compound appears to exert its effects by interrupting glutamate neurotransmission [15]. Riluzole has been shown to reduce the presynaptic release of glutamate [15] and also to affect some postsynaptic properties [62]. In one study, riluzole induced loss of righting reflex in rats and in smaller doses it also reduced the MAC of halothane [42]. The inability of riluzole to bind at glutamate receptors [70] suggests that the hypnotic state induced by this agent is different from that produced by ketamine, which is known to be an NMDA receptor antagonist [72]. Interestingly, riluzole protects against glutamate-induced neural injury following ischaemia [41] and this may also provide a further possible use for this novel compound in neurosurgical intensive care. Further investigation of the anaesthetic mechanisms, possibly mediated by altered excitatory amino acid transmission, is certain to follow the initial observations on riluzole action.

Uptake. When glutamate is released from nerve endings, its actions are terminated by uptake into the surrounding glial cells and neurones. In addition, glutamate uptake occurs in the nerve terminal where it is recycled to glutamine [33]. This carrier also transports aspartate into the nerve terminal.

Uptake of aspartate was not affected by thiopentone, methohexitone, ketamine or urethane in a rat brain slice preparation [52]. Similar findings were found in rat thalamic slices using anaesthetic doses of thiopentone, methohexitone, urethane and ketamine [37]. Pentobarbitone, in the same study, did reduce slightly the uptake of aspartate but the authors concluded that this effect was unlikely to contribute to the mechanism of action of the barbiturates.

At present, there is little evidence to link the excitatory amino acid neurotransmitters to anaesthetic mechanisms, despite their possible relevance to consciousness. More work in this area is required to clarify the significance of the anaesthetic effects reported to date [5, 32, 42].

Inhibitory transmitters: GABA and glycine

GABA. GABA is formed from the irreversible decarboxylation of glutamate, catalysed by the enzyme glutamic acid decarboxylase (GAD) (fig. 4). After release into the synapse, GABA transmission is terminated by uptake of the transmitter by surrounding glial cells and nerve terminals and this process, similar to that for glutamate, limits the activity of GABA in the synapse.

GABA is the major inhibitory neurotransmitter in the central nervous system [33]. By acting on both pre- and postsynaptic receptors, it causes an increase in chloride ion conductance which results in hyperpolarization of the nerve membrane. The barbiturate and steroid anaesthetic agents increase this GABA-stimulated chloride conductance, resulting in enhanced hyperpolarization [64], whereas the volatile anaesthetics have a weaker effect on the GABA receptor- Cl^- channel complex [54].

Catabolism of GABA. In a study using rat brain slices, it was found that 3% halothane had no effect on either the uptake or release of GABA [14]. However, the catabolism of GABA in the preparation

was reduced. This could lead to increased concentrations of GABA in the synapse. Similar effects of halothane on GABA catabolism have also been observed in synaptosomes prepared from rat brain [13]. In addition, clinical concentrations (1 MAC) of chloroform, enflurane and diethyl ether significantly affect GABA catabolism [13]. Of the barbiturates studied, only thiopentone inhibited GABA catabolism at clinical concentrations: pentobarbitone and phenobarbitone had effects only at very large doses [13]. The different actions that have been described, namely reduction in GABA catabolism by the volatile anaesthetic agents and interaction with the GABA receptor- Cl^- channel by the barbiturates, illustrate the possibility that different classes of anaesthetic agents may achieve the same physiological effect via different pharmacological mechanisms.

GABA uptake. No effect was found on the uptake of GABA into rat brain slices in the presence of thiopentone, methohexitone, Althesin, ketamine, halothane or urethane [52]. Similarly, pentobarbitone had no effect in rabbit hippocampal slices [34], or thiopentone, methohexitone, urethane and ketamine in rat thalamic slices [37]. It would appear that the uptake process does not play a significant role in the anaesthetic action of these compounds.

GABA release. Potassium-stimulated GABA release from rat brain slices has been shown to be reduced by the i.v. barbiturates, hydroxydione and pentobarbitone [52]. However, in the rat olfactory cortex preparation, there appeared to be an increase in the release of GABA in the presence of pentobarbitone [19]. In a third study, a biphasic response was observed in the rat thalamus slice preparation, in which small concentrations of thiopentone and methohexitone produced enhanced release, and larger concentrations reduced release [37]. The contrasting effects of barbiturates on GABA release from different brain regions probably reflect the complexity of action of these agents such that it is the balance of the effect on excitatory and inhibitory transmission that results in net excitatory or inhibitory synaptic events. Results obtained from slice preparations may be complicated by secondary cellular cross-talk, and the synaptosome preparation may be more useful for distinguishing primary effects at the level of the GABA releasing terminal.

The conflicting results concerning the effects of anaesthetic agents on the release of GABA from different areas of the brain *in vitro* [19, 37, 52, 68], necessitate caution in the extrapolation of observations to the whole animal. In one study, the effect of halothane on the concentration of GABA release in an *in vivo* preparation was investigated [57]. A microdialysis technique was used in which catheters were placed into the extracellular fluid (ECF) surrounding the brain and the concentrations of neurotransmitters measured. A reduction in GABA content of the ECF was observed in the animals anaesthetized with halothane. Although it was not possible to tell if this reduction was a result of a decrease in release or an increase in uptake, this technique did indicate alteration in transmitter homeostasis in the intact animal.

In summary, the evidence points to a number of potential sites of action of anaesthetic agents on the GABA transmitter system, but as in other transmitter systems, further work is required.

Glycine. Glycine is the major inhibitory neurotransmitter in the spinal cord and medulla and is also implicated widely in other brain regions. This transmitter acts on a receptor-operated chloride channel complex, similar to the GABA receptor- Cl^- complex [38]. The effects of anaesthetics on glycine transmission have not been studied extensively, but one study on rat brain slices has indicated that pentobarbitone has no effect on the potassium-stimulated release of glycine [68]. Further study is necessary to investigate the possible role of glycine transmission in anaesthetic mechanisms.

GROUP 2—CATECHOLAMINES, ACETYLCHOLINE AND 5-HT

Noradrenaline and adrenaline

The characteristics of release of noradrenaline from peripheral nervous tissue have been documented widely, owing to the availability of the chromaffin cell preparation derived from bovine adrenal medullary glands. This cultured cell preparation has been used as a model to investigate the action of anaesthetics on the process of neurosecretion [1, 53, 58, 59]. While contributing to the understanding of anaesthetic mechanisms and, in particular, to their effect on stimulus-secretion coupling, it should be remembered that this is only a model of the mechanisms in the CNS.

The barbiturates, at clinical concentrations, have been shown to reduce both the potassium- and carbachol-stimulated release of noradrenaline from chromaffin cell preparations [58], and from potassium-stimulated synaptosomes [31]. When the volatile anaesthetic agents halothane (fig. 5), enflurane, methoxyflurane and isoflurane were studied, a similar reduction in adrenaline and noradrenaline

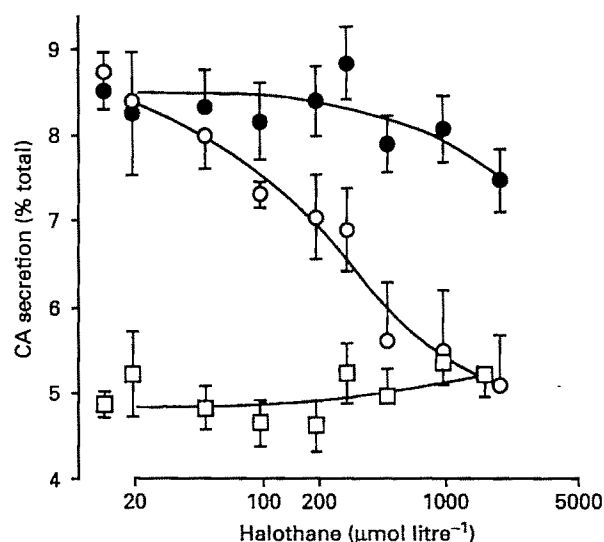


FIG. 5. Dose-response relationship for the effect of halothane on catecholamine (CA) secretion from bovine chromaffin cells. $\square$ = Basal secretion; $\circ$ = after stimulation with carbachol $500 \mu\text{mol litre}^{-1}$; $\bullet$ = after stimulation with potassium $77 \text{ mmol litre}^{-1}$ (reproduced with permission from [59]).

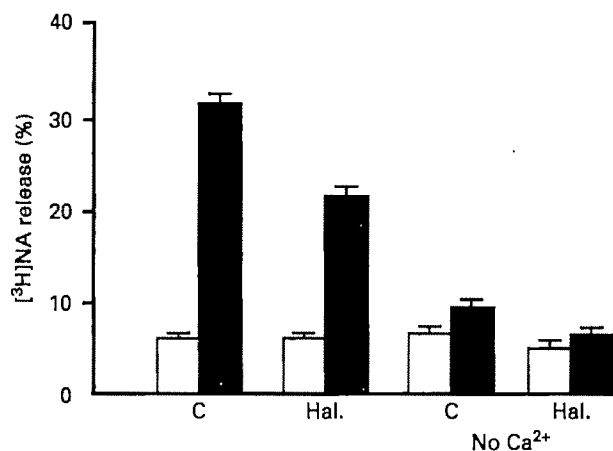


FIG. 6. [^3H]Noradrenaline (NA) release from rat slice preparation in the absence (C) and presence of 1.25% halothane (Hal.). Release stimulated with buffer containing potassium $28 \text{ mmol litre}^{-1}$. Diagram also demonstrates the smaller release in a calcium free buffer. $\square$ = Control release; $\blacksquare$ = potassium-stimulated release. Each point is the mean (SEM) of at least three experiments (reproduced with permission from [8]).

release was seen when carbachol stimulation was used. However, in contrast to the barbiturates, much larger doses of the volatile anaesthetic agents were needed to inhibit the release after potassium-stimulation. Again, evidence from this experimental system indicates possible selectivity for different classes of anaesthetic agents. However, this apparent selectivity could simply reflect the ability of different anaesthetic agents to reduce calcium concentrations in cells [58, 59] (see below). In contrast to observations on chromaffin cells, reduced potassium-evoked release of noradrenaline has been observed in rat brain slice preparations in the presence of volatile anaesthetic agents at clinical doses (fig. 6) [7, 8]. When applied at equipotent concentrations for producing loss of righting reflex in rats, halothane, enflurane and methoxyflurane were found to reduce potassium-stimulated noradrenaline release by 30% [7].

It has been suggested that anaesthetic agents may increase the ionic permeability of synaptic vesicles and thereby lead to a decrease in the proton gradients that are essential for synaptic vesicles to accumulate transmitters [6]. This "pump leak" hypothesis has been investigated for noradrenaline in the chromaffin cell system. Halothane, diethyl ether, butanol and ethanol were found to cause only a small insignificant loss of noradrenaline from chromaffin granules [1], inconsistent with the pump leak hypothesis. By inference, it appears unlikely that depletion of noradrenaline or other neurotransmitters from secretory vesicles could lead to loss of efficiency of synaptic transmission and subsequent anaesthetic state. However, taken together, *in vitro* observations for noradrenaline and adrenaline release suggest susceptibility of stimulated release mechanisms to modification by anaesthetic agents.

Complimentary studies have been performed *in vivo*. By manipulation of central noradrenaline concentrations, it has been shown that the MAC of halothane increases with increasing concentrations and decreases with decreasing concentrations, sug-

gesting that reduced noradrenaline transmission may be an important contributory factor to the induction of the anaesthetic state [50]. Thus observations from both *in vitro* and *in vivo* investigations indicate that central noradrenaline metabolism is worthy of further study in unravelling the actions of anaesthetic drugs.

Dopamine

Dopamine has a better understood and more localized central action than noradrenaline. As a result, Parkinson's disease and schizophrenia may both be treated using drugs that manipulate central dopamine metabolism. It is noteworthy that amphetamine, which leads to a state of arousal, stimulates dopamine release centrally. By inference, therefore, the dopaminergic system is a potential target for anaesthetic action.

Concentrations of dopamine in the CNS can be manipulated by either increasing concentrations of the dopamine precursor, L-dihydroxyphenylalanine (L-Dopa), to increase concentrations, or by depleting dopamine using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is an analogue of pethidine that selectively kills central dopaminergic neurones by inhibiting respiratory chain enzymes via a metabolic product. When mice were subjected to these two treatments, L-Dopa decreased the MAC of halothane and MPTP treatment increased the MAC of halothane [65]. Furthermore, when the concentrations of dopamine of MPTP-treated mice were repleted with L-Dopa, the MAC of halothane was restored to control levels.

In a second complex study, using an *in vivo* microdialysis technique, the effect of halothane anaesthesia on dopamine secretion was investigated [57]. Halothane was found to increase basal concentrations of dopamine when rats were anaesthetized. Results from both these studies conflict with expectations arising from the effects of amphetamine on dopamine metabolism. *In vivo* and *in vitro* studies are urgently required to characterize the effects of anaesthetic agents on dopamine metabolism in more detail. In view of the number of patients with alteration in central dopamine pharmacology, an enhanced understanding of anaesthetic mechanisms in the dopaminergic system is likely to have important clinical implications.

5-HT

A precise functional role for 5-HT has yet to be fully established. However, 5-HT has been implicated as an important transmitter in the feeding, sleep, mood, behaviour and cardiovascular control pathways [3].

5-HT is synthesized from the amino acid L-tryptophan in a two-step pathway. The first, a rate limiting step, involves conversion of L-tryptophan to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. This is followed by decarboxylation of 5-hydroxytryptophan to 5-HT, catalysed by L-aromatic acid decarboxylase. L-tryptophan is transported actively into the nerve terminal and this may be a further limiting factor in the synthesis of 5-HT. After stimulated release into the synapse, the

postsynaptic response is terminated by removal of 5-HT from the cleft by a high-affinity active transport mechanism.

To date, only two aspects of 5-HT metabolism have been investigated in the presence of anaesthetic agents, 5-HT uptake and release. There have been no studies on the effects of anaesthetic agents on the uptake of L-tryptophan, or on the rate limiting enzyme tryptophan hydroxylase, which may represent important control points in this transmitter system.

5-HT uptake. In a series of experiments using the rat synaptosome preparation, ketamine, enflurane, halothane and isoflurane [43–45, 47] inhibited the uptake of 5-HT into the synaptosomal fraction. It is possible that inhibition of uptake may increase the effect of the transmitter at its postsynaptic receptor site as the synaptic activity of 5-HT is terminated by an active re-uptake mechanism. 5-HT is believed to exert its influence on analgesic mechanisms by activating inhibitory mechanisms in the dorsal horn of the spinal cord, hence a decrease in re-uptake may contribute to the anaesthetic effects of these drugs. In contrast, during the same series of experiments, pentobarbitone and etomidate were found to have little effect on the activity of the 5-HT uptake process, again suggesting selectivity for anaesthetic agents on different neuronal activities.

To further investigate the selectivity of action of anaesthetic agents on the 5-HT uptake mechanism, competition binding studies have been carried out using [^{3} H]paroxetine, which binds competitively to the substrate recognition site of the 5-HT transporter [46]. Ketamine caused a concentration-dependent inhibition of specific [^{3} H]paroxetine binding, whereas halothane had no effect. Interestingly, ketamine and halothane were found to induce a similar level of inhibition of 5-HT uptake at clinically relevant concentrations of anaesthetic agents. Thus while ketamine may cause inhibition of 5-HT uptake by direct interaction with the active site of the transport protein, inhibition of uptake by halothane must be mediated, at the very least, by a distinct site on the transporter protein or possibly by mechanisms located elsewhere in the membrane.

Ethanol in sufficient quantities causes general anaesthesia in humans [2]. When an equivalent dose of ethanol was applied *in vitro* to a synaptosome preparation, the uptake of 5-HT was stimulated [2], in contrast to other anaesthetic agents studied above [43–47]. These contrasting effects on the 5-HT transmitter uptake mechanism do not offer a simple explanation for the possible role of this process in anaesthesia.

5-HT release. To date, only the effect of pentobarbitone on 5-HT release from rat midbrain slices has been studied. A small decrease (20%) in 5-HT

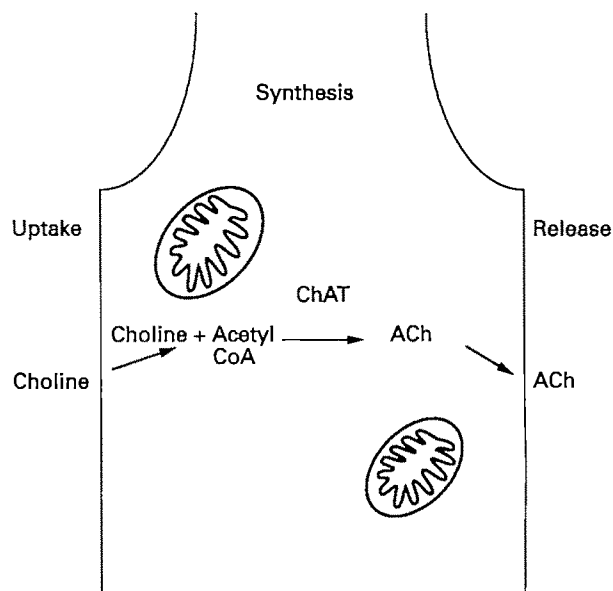


FIG. 7. Diagrammatic representation of acetylcholine (ACh) metabolism in presynaptic nerve terminals. ChAT = Choline acetyltransferase.

duction in the release of 5-HT, which was not present in the halothane anaesthetized group. Further experiments suggested that the reduction in 5-HT release observed after application of acetylcholine may be a result of stimulation of GABA interneurons. This work suggests that 5-HT concentrations could vary in response to anaesthetic agents, because of indirect effects on other transmitter systems, and demonstrates the complex interconnections between neurotransmitter systems that are seen in whole animal preparations which cannot be studied easily in *in vitro* systems.

Acetylcholine

Acetylcholine is well established as an excitatory neurotransmitter. A number of *in vivo* observations have suggested that cholinergic transmission may be a target for anaesthetic action. In a classical study on acetylcholine transmission, the release of acetylcholine from the surface of the rabbit brain was shown to decrease during barbiturate anaesthesia and increase after a period of feeding and walking, indicating a role for acetylcholine in the maintenance of consciousness [18]. Clinical anaesthetists will be aware also of the prolonged sedative effects that anticholinergic drugs produce, particularly in the elderly population [66]. In a recent study, central cholinergic activity was shown to oppose ketamine anaesthesia [51] and this effect on ketamine anaesthesia was antagonized when the anticholinergic drug 1-hyoscyamine, was given as premedication. These lines of evidence emphasize the important role of acetylcholine in the control of conscious level.

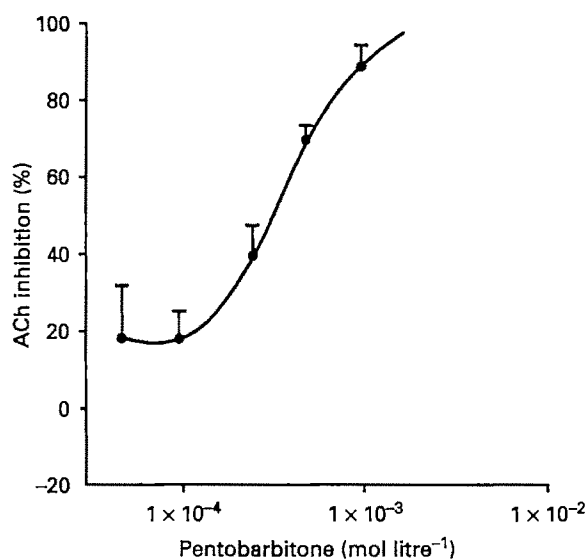


FIG. 8. Effect of pentobarbitone on potassium-stimulated acetylcholine (ACh) release from rat midbrain slices. Values are mean (SEM) of 3–4 determinations (reproduced with permission from [61]).

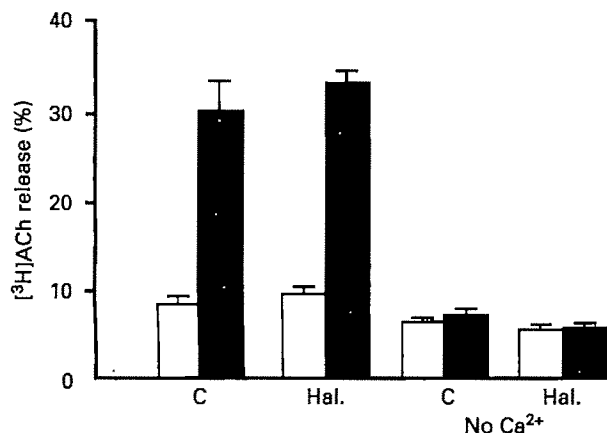


FIG. 9. $[^3\text{H}]$ Acetylcholine release from rat slice preparation in the absence (C) and presence of 1.25% halothane (Hal.). Release stimulated with potassium $16 \text{ mmol litre}^{-1}$. Figure also shows smaller release in a calcium free buffer. $\square$ = Control release; $\blacksquare$ = Stimulated release. Each point is the mean (SEM) of 1–4 experiments, each in triplicate or quadruplicate (reproduced with permission from [8]).

probably the uptake of choline into the nerve terminal, making this stage a candidate for the action of anaesthetic agents [36].

Acetylcholine metabolism. The effect of halothane on the uptake of $[^3\text{H}]$ methylcholine has been investigated in rat brain synaptosomes [28, 35]. A 40% reduction in the uptake of the precursor was demonstrated at halothane concentrations of 2.4 MAC. A similar reduction was seen also when enflurane and isoflurane were studied. Whether the action of the volatile anaesthetics on precursor uptake is competitive [35] or non-competitive [28] has yet to be resolved. As choline uptake is rate limiting [36] for the biosynthesis of acetylcholine, the inhibition of precursor uptake mediated by the volatile anaesthetics may contribute to the state of anaesthesia.

Turnover of acetylcholine *in vivo* in rat brain regions has been measured under halothane, en-

flurane and ketamine anaesthesia [55]. The concentrations of acetylcholine and choline in various brain regions did not alter during anaesthesia with any agent. However, halothane decreased the turnover rate for acetylcholine by 10% at 0.7–1.0% inspired concentrations in both cortical and subcortical structures. Interestingly, enflurane did not show any decrease in acetylcholine turnover in subcortical structures and it is possible that this may be associated with the seizure-like activity that develops under enflurane anaesthesia [53]. Unlike halothane and isoflurane which had no effect at 2.4 MAC, enflurane has been shown to alter ChAT activity in rat brain synaptosomes [29] by reducing the Michaelis constant (K_m) of the enzyme for choline. *In vivo*, such an effect could potentiate the synthetic pathway for acetylcholine and contribute to the central effects on the electroencephalogram [53] and to the seizure activity that may develop with large inspired concentrations of enflurane. This may also explain the maintenance of acetylcholine concentrations in subcortical structures under enflurane anaesthesia in the *in vivo* study described above [55].

Acetylcholine release. Few studies of the effects of anaesthetic agents on release of acetylcholine have been performed. In rat slice preparations, potassium-stimulated acetylcholine release has been shown to be decreased in the presence of pentobarbitone (fig. 8) [60, 61, 68] and methohexitone [61]. Similarly, halothane, at a concentration of 2.5 MAC, has been shown to reduce potassium-stimulated release of acetylcholine by approximately 50%, from a rat brain synaptosome preparation [35]. A reduction in acetylcholine release has also been demonstrated with 1 and 2% halothane in the cat superior cervical ganglion preparation [12]. However, conflicting results have been obtained in other studies. Halothane, methoxyflurane and enflurane, at concentrations of 1.25%, 0.2% and 3%, respectively, were reported to have no effect on acetylcholine release from a rat brain slice preparation (fig. 9) [7, 8] and in an isolated cat superior cervical ganglion preparation, chloral hydrate was found to either decrease potassium-stimulated release [61] or to have no effect [48].

In the above studies, total acetylcholine release was measured in synaptosomes [35], while release of $[^3\text{H}]$ acetylcholine was monitored in the brain slice studies [7, 8]. Differential labelling of intracellular pools of acetylcholine in the nerve terminal [71] could underlie some of the differences in the observations [7, 8, 35]. In addition, rat brain slices are known to be more sensitive to potassium depolarization than synaptosomes [63] and this could also account for differences in actions of anaesthetics between the preparations. Comparisons between these studies may be further complicated by the different doses of anaesthetic agents applied in the various studies [8, 35].

OTHER TRANSMITTERS

A number of other transmitter systems, in particular those classified as group 3, including neuropeptides such as substance P, the opioids and thyrotrophin

releasing hormone (TRH), have received little or no attention with respect to the effects of anaesthetics. At present, 40 neuroactive peptides have been identified, but not all satisfy the criteria for neurotransmitters [38]. Therefore there remains a large amount of work before questions regarding selectivity of anaesthetic action can be answered fully.

THE ROLE OF CALCIUM IN NEUROTRANSMITTER RELEASE: EFFECT OF ANAESTHETIC DRUGS

Influx of extracellular calcium is essential for the coupling of electrical excitation to neurotransmitter release [17]. Two phases of depolarization-induced calcium entry into the nerve terminal have been characterized, a rapid phase and a slower plateau phase [40]. The release of different neurotransmitters may rely on different phases of the calcium current [40]. As calcium is crucial to neurotransmitter release, this aspect of stimulus-secretion coupling may be a potential site of action of anaesthetic agents and may present a mechanism by which some selectivity of anaesthetic action may occur.

Depolarization-induced calcium uptake into synaptosomes has been shown to be reduced in the presence of a wide range of anaesthetic agents [10, 21, 30]. Furthermore, in bovine chromaffin cells, anaesthetic-induced reduction in calcium influx has been linked to a decrease in evoked noradrenaline release in the presence of pentobarbitone, halothane (fig. 10), isoflurane, enflurane and methoxyflurane [58, 59, 73]. When noradrenaline release from chromaffin cells was stimulated with carbachol or potassium, carbachol-evoked release appeared to be more susceptible to the action

of clinical doses of volatile anaesthetic agents than potassium-evoked release. Inhibition of potassium-stimulated release was affected only at supraclinical doses of anaesthetic agents. These results are most likely to be explained by the concentration of calcium in the cell achieved after the two stimulation procedures. When stimulated with potassium, the influx of calcium was much greater than when stimulation was with carbachol, thus halothane-induced reduction in intracellular calcium concentrations to threshold concentrations for transmitter release was more easily achieved under carbachol stimulation. These results are consistent with an important role for calcium homeostasis in anaesthetic-induced modulation of neurotransmitter release.

The possibility that reductions in calcium influx in the presence of anaesthetic agents may be linked to the membrane disordering properties of these drugs has been investigated further [21, 30]. No simple relationship between the ability of anaesthetic agents to reduce calcium influx and disorder membrane structure was shown. A stronger correlation was found between the effect on sodium influx and membrane perturbation [30].

DIRECT VERSUS INDIRECT ACTIONS OF ANAESTHETIC AGENTS ON PRESYNAPTIC TRANSMITTER MECHANISMS

So far in this discussion, consideration of anaesthetic effects at the level of the presynapse has focused on specific aspects of synaptic transmission mediated by a variety of transmitter agents. A variety of effects have been reported, some of which may be compatible with the onset of anaesthesia. An important question that remains is how the anaesthetic agents exert their effects on such a range of biochemical systems.

From a pharmacological perspective, most general anaesthetic agents would appear to be essentially non-specific in their actions at the level of transmitter metabolism and release. However, as can be seen from the above discussion, not all anaesthetics produce the same response in the assay systems exploited, indicating some degree of selectivity of action (see tables I and II). Indeed, it is possible that certain anaesthetic agents are found to exert their primary effects in a specific manner at the level of discrete proteinaceous acceptors, for example ketamine acts at the NMDA receptor [51] and at the active site of the 5-HT transporter [46], the barbiturates act at the GABA receptor-Cl⁻ complex [65] and isoflurane produces stereoselective activation of a potassium channel subtype [26]. However, when agents such as the volatile anaesthetic agents are considered, a wide spectrum of effects in different transmitter systems is observed, involving not only presynaptic but also postsynaptic events. In these cases, anaesthetic effects may reflect the susceptibility of discrete cellular functions to non-specific, anaesthetic-mediated changes in the local environment rather than specific interactions with proteinaceous acceptors. Hence, anaesthesia may be the net result of a combination of a large number of non-specific toxicological effects on CNS function that

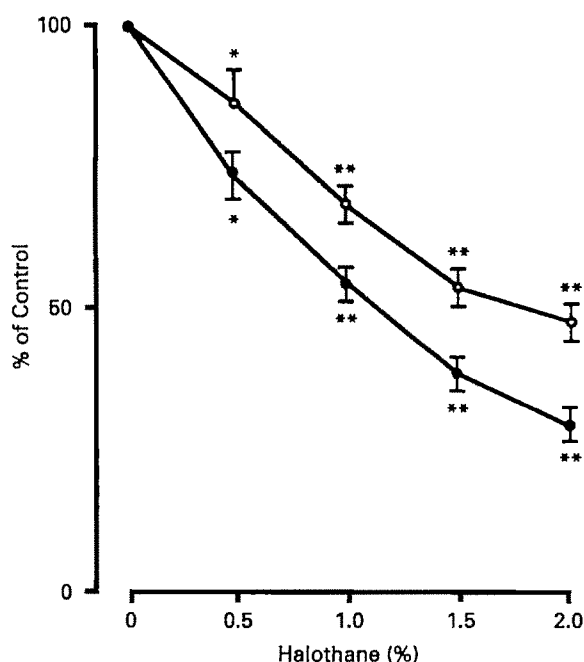


FIG. 10. Effects of halothane on secretion of catecholamines (○) and influx of ⁴⁵Ca (●) evoked by carbachol in cultured bovine adrenal medulla cells. Values are mean (SEM) from 10 separate experiments, expressed as a percentage of carbachol-evoked responses in the absence of halothane. * $P < 0.05$, ** $P < 0.01$ (reproduced with permission from [73]).

TABLE I. *Effect of volatile anaesthetic agents on neurotransmitter release.* ↑ = Increased, ↓ = decreased, → = no change in transmitter release

Volatile agent	Excitatory amino acids	Acetylcholine	Noradrenaline	Dopamine
Halothane	[5]→, [32]↑	[7], [8]→, [35], [12]↓	[1], [7], [8], [59]↓	[57]↑
Enflurane	[32]↑	[7]→	[7], [8], [59]↓	
Isoflurane			[59]↓	
Methoxyflurane		[7]↓	[7], [8], [59]↓	
Diethyl ether	[32]→		[1]↓	

TABLE II. *Effect of i.v. anaesthetic agents on neurotransmitter release.* ↑ = Increased, ↓ = decreased, → = no change in transmitter release

Drug	Excitatory amino acids	GABA	Acetylcholine	Noradrenaline
Pentobarbitone	[19], [52], [58], [68]↓	[19]↑, [52]↓, [68]↓	[60], [61], [68]↓	[31], [58]↓, [68]↓
Thiopentone	[37]↓ + ↑, [52]↓			
Methohexitone	[37]↓ + ↑, [52]↓		[61]↓	
Althesin	[37]→, [52]→			
Hydroxydione	[52]↓			

any particular agent exerts. If so, the “physiological mechanism” of anaesthesia may well be different for each agent.

At the present time, there have been insufficient studies to allow any definitive statements to be made concerning the relevance of the action of anaesthetic agents on presynaptic transmitter release mechanisms to the overall mechanisms of general anaesthesia (see tables I and II). In many cases, our knowledge of the effects of anaesthetic agents on discrete presynaptic activities depends on one or two studies. Often these have been carried out on more than one experimental system and under different experimental conditions, such that a true comparison of results is not possible. More systematic studies of the actions of general anaesthetic agents on individual presynaptic activities in defined experimental systems and under comparable conditions are urgently required. Furthermore, these should include both *in vitro* and *in vivo* studies to allow primary and secondary effects of anaesthetic agents to be dissected. While *in vitro* studies may allow effects on specific functions to be studied more easily, the importance of *in vivo* studies must be stressed to permit the discrimination of statistically significant changes measured *in vitro* which have no physiological relevance *in vivo*. Since a universal action of general anaesthetic agents cannot be ruled out, one aim of these studies should be to identify central activities that are universally altered by general anaesthetic agents in a manner consistent with the induction of the anaesthetic state. The likelihood is, however, that physiological mechanisms of anaesthesia will prove to be much more complex.

The feature that unifies all general anaesthetic agents is their relatively high hydrophobicity. Indeed, lipid solubility is the only property of all anaesthetic agents that appears to correlate with anaesthetic potency [49]. Hence, anaesthesia may depend solely on the ability of any agent to disrupt local hydrophobic environments such that central mechanisms in the control of consciousness become

disrupted. Selectivity of agents may, therefore, be related to their ability to penetrate hydrophobic domains of different central functional structures [23]. It is likely that many of the effects of anaesthetic drugs will be mediated via their disordering effects in the lipid bilayer of the cell membrane [49]. Where anaesthetic effects on membrane functions are shown, it will be important to investigate the possible relationship of these changes with alterations in the membrane environment.

When individual central activities are considered, the anaesthetic agents appear not to act in concert but to exert some selectivity. For example, the barbiturates exert different effects on excitatory amino acid transmitter release than the volatile anaesthetics [5, 19, 32, 37, 52]. Further study of presynaptic transmitter systems may allow agents to be grouped functionally which, in turn, may simplify the investigation of their mechanisms.

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ALPHA₂ ADRENOCEPTOR AGONISTS AND ANAESTHESIA

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The clinical application of alpha₂ adrenergic agonists for anaesthesia has no precedent in our discipline. New classes of anaesthetic drugs, or even new members of an existing class, are usually promoted by an immense pharmaceutical industry effort. The alpha₂ adrenergic agonists have arrived on the scene with no hyperbole: these drugs will succeed (or fail) on their own merits. In addition, more is known about the underlying mechanism of action of these compounds than is known about all other anaesthetic agents. In this review we have concentrated on basic, applied and clinical pharmacology of these agents as they relate to anaesthesia.

BASIC PHARMACOLOGY

Classification of adrenergic receptors

Until recently, the framework for the understanding of adrenergic responses was based on the dual receptor theory of Ahlquist [5]. He differentiated adrenergic receptors into alpha and beta based on the rank order of potency of various natural and synthetic catecholamines in different physiological preparations. Ahlquist concluded that there was no clear correlation between excitatory or inhibitory actions of catecholamines and a particular receptor type. Activation of either alpha or beta adrenergic receptors produced excitatory effects in some tissues and inhibitory effects in others. Subsequently Lands and colleagues [86] showed that the rank order of potency of a series of catecholamines for a variety of beta adrenergic receptor-mediated responses could be used to identify two types of beta adrenergic receptors, beta₁ and beta₂. Both the beta₁ and the beta₂ adrenoceptors stimulate the membrane-bound enzyme adenylate cyclase, leading to the intracellular accumulation of cyclic adenosine 3', 5'-monophosphate (cAMP), a ubiquitous second messenger present in virtually all cells and tissues [140]. The beta adrenoceptors have become model systems for investigators studying the structure and function of hormone and drug receptors in cell membranes because of their ubiquity, close coupling to a biochemical effector unit and the widespread clinical application of ligands acting at these receptors [42, 88, 137].

In the alpha adrenoceptor field, the next major advance was based on the identification of a receptor which regulated the release of neurotransmitters [108]: from this it was inferred that the receptor was located at a presynaptic site [87]. This led to a subdivision of alpha adrenoceptors, based on their synaptic location, into postsynaptic alpha₁ and presynaptic alpha₂ [14]. A classification based strictly on anatomic location has proven to be untenable in the light of postsynaptically and even extra-synaptically located alpha₂ adrenergic receptors not linked to neurotransmitter release [44]. As more selective alpha adrenoceptor antagonists became available, it was possible to definitively separate the alpha adrenoceptors into two subtypes on a pharmacological basis [24]. The current pharmacological classification of alpha₂ vs alpha₁ is based on the antagonists yohimbine and prazosin. At alpha₁ receptors, prazosin is more potent than yohimbine, whereas at alpha₂ receptors, yohimbine is more potent than prazosin.

Structure of the alpha₂ adrenoceptor

The structure of the alpha₂ adrenoceptor is similar to a host of other neurotransmitter receptors, including other adrenergic (alpha₁, beta), muscarinic, dopamine, opioid, adenosine and serotonin receptors. Each of these receptor proteins is comprised of a single polypeptide chain which weaves back and forth through the cell membrane. The hydrophobic intramembranous portions of each of the adrenergic receptors are similar in their primary structure. From this, one may deduce that these hydrophobic portions are probably the site which recognizes noradrenaline, the ubiquitous neurotransmitter for each of the adrenergic receptors. However, on the cytoplasmic side, the adrenergic receptor proteins exhibit considerable differences in structure. The characteristic adrenergic responses are predicated by these structural features, especially in the manner in which they provide "contact points" for the host of guanine nucleotide binding proteins (G proteins).

Classification of alpha₂ adrenergic receptors

Two separate nomenclatures, based on either pharmacological (alpha_{2A}, alpha_{2B} or alpha_{2C}) or

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molecular biological probes [23] agree to the existence of at least three different alpha₂ isoreceptors. According to the molecular biological classification, these are defined by the chromosomal location of the gene for the receptor, being either alpha_{2C2}, alpha_{2C4} or alpha_{2C10}. Different brain regions, like most other tissues in the body, are usually populated by more than one isoreceptor.

G proteins

These "coupling proteins" promote transmembrane signalling to a discrete effector mechanism which may be a transmembrane ion channel or an intracellular second messenger cascade [29]. There are more than 20 species of G proteins which are characterized by differences in the amino acid sequence of one (alpha) of the three subunits. These discrete differences in the alpha subunit provide the unique response mediated by each of the adrenergic receptors. At least four different G proteins couple to the alpha₂ adrenoceptors, including G₁₋₃ and G_o.

Effector mechanisms

A feature common to all alpha₂ adrenergic receptors is their ability, when activated, to inhibit adenylate cyclase. The resulting decrease in the accumulation of cAMP attenuates the stimulation of cAMP-dependent protein kinase and hence the phosphorylation of target regulatory proteins. However, in many cases a decrease in cAMP production is not sufficient to mediate the effects of the alpha₂ adrenoceptor. Efflux of K⁺ through an activated channel can hyperpolarize the excitable membrane and provide an effective means of suppressing neuronal firing. Alpha₂ adrenoceptor stimulation also suppresses Ca²⁺ entry into the nerve terminals which may be responsible for its inhibitory effect on secretion of neurotransmitters.

APPLIED PHARMACOLOGY

Alpha₂ adrenergic agonists

These can be grouped into three main classes: phenylethylamines (e.g. amethylnoradrenaline), the imidazolines (e.g. clonidine) and oxaloazepines (e.g. azepexole).

Clonidine, an imidazole compound, is a selective agonist for alpha₂ adrenoceptors with a ratio of 200:1 (alpha₂:alpha₁). In many models of alpha₂ action, clonidine has been identified as a partial agonist. Clonidine is rapidly and almost completely absorbed after oral administration and reaches peak plasma concentrations within 60–90 min by this route. Clonidine may also be delivered via a time release transdermal patch, although a minimum of 2 days must elapse before a therapeutic concentration is achieved [144]. The elimination half-life of clonidine is between 9 and 12 h, and approximately 50 % of the drug is metabolized in the liver to inactive metabolites while the rest is excreted unchanged by the kidney.

Methyldopa is metabolized to methylnoradrenaline which is a full agonist at the alpha₂ receptor and has 10-fold selectivity for the alpha₂ over the alpha₁

adrenoceptor. Because transformation into the active compound is necessary, effects are slow to develop (4–6 h) and somewhat unpredictable. It is the only parenteral preparation available for clinical use in the U.S.A. Guanabenz is similar to clonidine in its effects: however, it is less potent and shorter acting with a terminal elimination half-life of 6 h. Guanfacine has the longest half-life (14–18 h) of all the clinically available alpha₂ agonists. Both the latter two drugs are guanidine compounds.

Medetomidine (4(5)-[1,2,3-dimethylphenylethyl]-imidazole) is the prototype of the novel super-selective alpha₂ agonists. It is an order of magnitude more selective than clonidine and is a full agonist at this class of receptor [129]. Medetomidine is extremely potent and is active at small nanomolar concentrations and has been widely used in veterinary practice in Europe. Since the D-enantiomer of this racemate is the active ingredient, dexmedetomidine has been developed for clinical use. Phase II studies with this compound have been launched in the U.S., while in Europe the drug is in phase III clinical trials.

Some ligands have an imidazole ring which enables them to bind to non-adrenergic imidazole-preferring receptors, as well as to the alpha₂ adrenoceptor [51]. The cardiovascular properties of alpha₂ ligands vary considerably, depending on whether or not the imidazole-preferring receptor is also bound [142].

CLINICAL APPLICATION OF ALPHA₂ AGONISTS

Pharmacological responses of alpha₂ agonists on different systems

Central nervous system. Sedation is one of the most consistent effects mediated by central alpha₂ receptors [43]. While this property is an undesirable side effect when clonidine is administered to patients with hypertension, it has been used to great advantage as premedication in anaesthesia. This sedative effect of alpha₂ agonists is potentiated significantly when they are administered together with a benzodiazepine [126]. Recently, the locus coeruleus was shown to be a principal region responsible for the sedative effect [35, 127].

Another characteristic effect of alpha₂ agonists in clinical situations is anxiolysis, which is comparable to that produced by benzodiazepines [26, 54, 153]. Clonidine may also depress panic disorder in humans [147]. However, larger doses of alpha₂ agonists may produce anxiogenic responses through non-selective activation of alpha₁ receptors [133].

Alpha₂ adrenergic receptor activation produces a potent analgesic response, involving both supra-spinal and spinal sites [110, 151, 154]. In animal experiments, clonidine exerts a more potent analgesic effect than morphine [55]. Furthermore, its potency is enhanced synergistically with concomitant treatment with opioids [103, 105, 134, 152]. Alpha₂ agonists and opioids mediate the analgesic action through independent receptors, although these two classes of agents have a similar transduction pathway in their effector mechanism [22]. This may be the mechanism for the development of cross-tolerance between these two agents [107, 136]. Alpha₂ agonists

suppress the undesirable physiological and psychological symptoms after withdrawal of opioids [62]. Recently, the usefulness of α_2 agonists has been extended to other withdrawal states, such as alcohol and benzodiazepines [10, 36, 155]. In humans, dexmedetomidine was reported to suppress ischaemic pain [76] and to attenuate the affective component of ischaemic pain [82]; yet, i.v. dexmedetomidine, in the dose range 25–50 $\mu\text{g kg}^{-1}$, did not affect the experimental pain threshold [76].

The most impressive action of α_2 agonists in the central nervous system is the ability to reduce anaesthetic requirements. Kaukinen and Pyykko [81] demonstrated a modest reduction (15%) of MAC of halothane after subacute administration of clonidine in rabbits. Bloor and Flack [16] observed that acutely administered clonidine reduced MAC of halothane by up to 50% in a dose-dependent fashion. This effect was antagonized by tolazoline, an α_2 antagonist. There is a ceiling to the reduction of MAC effect of clonidine because of its ability to stimulate α_1 adrenoceptors. More selective α_2 agonists reduce the MAC of volatile anaesthetics to a much greater extent. Azeperole was shown to reduce MAC of isoflurane by 85% in dogs [94], while dexmedetomidine decreased MAC of halothane by more than 95% in animals [132, 149], indicating that it may be an anaesthetic in its own right. This reduction in anaesthetic requirement may also be demonstrated in humans and is not limited to volatile anaesthetics (see below).

Intraocular pressure can be reduced by α_2 agonists and these agonists may also attenuate the increase in intraocular pressure associated with laryngoscopy and tracheal intubation [60, 75, 85]. Although an early report showed that the action may be a result of reduction in production and augmentation in outflow of aqueous humour [83], this has yet to be established. The imidazoline preferring receptor has also been implicated in this action [113].

Experimental application of α_2 agonists and antagonists in the study of neuroprotection from cerebral ischaemia has resulted in conflicting data. Hoffman and colleagues [71, 72] reported that the α_2 agonists clonidine and dexmedetomidine improve outcome from incomplete global ischaemia. On the other hand, Gustafson and colleagues [65, 66] demonstrated that idazoxan, an α_2 antagonist, could also protect against global ischaemia. This apparent paradox may be reconciled by a recent report [92] that both idazoxan and rilmenidine (α_2 antagonist and agonist with affinity for the imidazole-preferring receptor) can exert a protective effect against cerebral ischaemia. The authors hypothesized that the imidazole-preferring receptor, and not α_2 receptors, are involved in the neuroprotective mechanism.

Cardiovascular system. The action of α_2 agonists on the cardiovascular system may be classified as peripheral or central. α_2 agonists inhibit noradrenaline release from peripheral prejunctional nerve endings and this property, in part, contributes to the bradycardiac effect of α_2 agonists [37]. At present, there is no evidence to support the existence of postsynaptic α_2 receptors in the myocardium

[45, 74]; therefore, direct effects of α_2 agonists on the heart are doubtful. Postjunctional α_2 receptors are present in both arterial and venous vasculature where they produce vasoconstriction [125]. Among the different vascular beds, the effect of α_2 agonists on the coronary circulation is important. A putative vasoconstrictive action of α_2 agonists on the coronary vasculature would not be favourable in the ischaemic heart [31, 97]; however, these agonists can ameliorate any direct constriction by reducing sympathetic outflow [70]. Furthermore, α_2 agonists have been documented to release endothelial derived relaxant factor in coronary arteries [32] and to enhance coronary blood flow induced by endogenous and exogenous adenosine in an *in vivo* model [73]. Thus the effect on coronary arteries may be too complicated to distinguish clear changes in coronary blood flow in an *in vivo* model [130].

Central mediated hypotensive and bradycardiac effects of clonidine have been well recognized. The mechanism for these actions may involve inhibition of sympathetic outflow and the potentiation of parasympathetic nervous activity. However, the precise mechanism involved in these actions is not well understood. While the nucleus tractus solitarius (a site known to modulate autonomic control, including vagal activity) is an important central site for the action of α_2 agonists [84], other nuclei, including the locus coeruleus [141], the dorsal motor nucleus of vagus [122, 148] and the nucleus reticularis lateralis [50, 143] may also mediate hypotension, bradycardia, or both. It has been documented [20, 21, 142] that imidazole-preferring receptors play an important role in the hypotensive effect of α_2 agonists.

α_2 agonists also have anti-arrhythmic properties. For example, dexmedetomidine prevents adrenaline-induced arrhythmias during halothane anaesthesia [68]. Not only central α_2 receptors, but imidazole-preferring receptors are involved [69]. The anti-arrhythmic effect is abolished in vagotomized animals [78].

The effect of α_2 agonists on the cerebral circulation during anaesthesia has been studied. Zornow and colleagues [156] and Karlsson and colleagues [80] demonstrated that dexmedetomidine decreases cerebral blood flow in dogs anaesthetized with isoflurane and halothane. This characteristic may be favourable in protecting the brain from an abrupt increase in blood flow. This idea has been supported by a recent report by McPherson and Traystman [91], who showed that dexmedetomidine blunts the cerebrovascular response to hypoxic hypoxia during isoflurane anaesthesia.

Respiratory system. The respiratory depressant effects of clonidine are not remarkable unless massive doses are given [8, 102]. Eisenach reported that i.v. clonidine induces an hypoxic effect in ungulates and concluded that aggregation of platelets is responsible for this mechanism in this animal species [47]. Although α_2 agonists may cause mild respiratory depression [98], the effect of clonidine is less than that of opioid narcotics [58]. In clinically appropriate doses, respiratory depression may not be detected

TABLE I. *Preanaesthetic application of alpha₂ adrenergic agonists*

Drug	Route (dose)	Effect	Reference
Clonidine	Oral (300 µg)	Sedation	[153]
		Reduction in anxiety	
		Decreased methohexitone	
Clonidine	Oral (200 µg)	Reduction in anxiety	[26, 28]
		Postoperative analgesia	
Clonidine	Oral (300 µg)	Sedation	[85]
		Reduction in anxiety	
		Reduction in intraocular pressure	
Clonidine	Oral (5 µg kg ⁻¹)	Decreased fentanyl by 45 %	[61]
		Decreased haemodynamic response to intubation	
Clonidine	Oral (200–300 µg)	Decreased sufentanil by 40 %	[57]
	+ n.g. (200–300 µg)	Haemodynamic stability	
		Decreased catecholamines	
Clonidine	Oral (5 µg kg ⁻¹)	Decreased droperidol	[49]
Clonidine	Oral (225–375 µg)	Decreased thiopentone by 20 %	[104]
Clonidine	Oral (600 µg)	Decreased propofol	[121]
Clonidine	Oral (150 µg)	Prolonged tetracaine spinal anaesthesia	[106]
Clonidine	Oral (5 µg kg ⁻¹)	Reduction in intraocular pressure	[60]
		Decreased haemodynamic response to intubation	
		Decreased isoflurane by 30 %	
		Decreased fentanyl by 60–75 %	
		Haemodynamic stability	
Clonidine	Oral (5 µg kg ⁻¹)	Decreased haemodynamic response to intubation	[59]
		Decreased isoflurane by 40 %	
		Decreased fentanyl by 75 %	
		Haemodynamic stability	
Clonidine	Oral (4.7 µg kg ⁻¹)	Decreased catecholamines	[115]
		Haemodynamic stability	
Dexmedetomidine	I.v. (0.6 µg kg ⁻¹)	Decreased isoflurane by 25 %	[7]
		Decreased haemodynamic response to intubation	
Dexmedetomidine	I.m. (1.0 µg kg ⁻¹)	Decreased thiopentone by 17 %	[1]
		Decreased catecholamines	

except with hypercapnic respiratory response studies [109]. There is no potentiation by clonidine of opioid-induced respiratory depression [11, 77]. In addition, nebulized clonidine attenuated bronchoconstriction in asthmatic patients [89].

Endocrine system. Alpha₂ agonists potentiate the secretion of growth hormone [64]. Although a precise mechanism for this action has not been elucidated, Devesa and colleagues [40, 41] suggested that alpha₂ receptor activation is coupled to growth hormone releasing factor. Alpha₂ agonists which possess an imidazole ring in their structure may produce inhibition of steroidogenesis. However, at clinical doses, this effect is not likely to have serious consequences [95]. Alpha₂ agonists decrease sympathoadrenal outflow and these agents may suppress the stress response after surgical stimulation [87]. While *in vitro* studies indicate that alpha₂ agonists regulate catecholamine secretion in the adrenal medulla [67, 150], this effect has been questioned by others [114, 120]. The alpha₂ agonists also inhibit the release of insulin from the pancreatic beta cells directly [9]; again, this effect is not troublesome in clinical situations [93].

Gastrointestinal system. Decreased salivary flow is one of the advantages of the use of alpha₂ agonists as premedication [79]. Alpha₂ agonists may modulate release of gastric acid via a presynaptic mechanism [14]; yet no significant change in gastric pH is observed in humans [104]. The alpha₂ agonists may also prevent intestinal ion and water secretion in the large bowel, indicating an effective treatment for watery diarrhoea [90].

Renal system. Alpha₂ agonists induce diuresis. Inhibition of release of antidiuretic hormone (ADH) [111], antagonism of the renal tubular action of ADH [135] and increase in the glomerular filtration rate [138] have each been implicated in this mechanism. Recently, alpha₂ agonist-induced release of atrial natriuretic factor has also been suggested to contribute to the diuretic mechanism of alpha₂ agonists [30].

Haematological system. Aggregation of platelets is induced by alpha₂ agonists [124]. In the clinical setting, this is largely offset by the decrease in circulating catecholamines.

Use of alpha₂ agonists in anaesthetic practice

Preanaesthetic administration (table I). Since sedation and anxiolysis are necessary attributes of premedication, administration of alpha₂ agonists suits this purpose well [1, 6, 7, 28, 85, 153]. Another benefit of alpha₂ agonists as premedication are their ability to potentiate the anaesthetic action of other agents and to reduce anaesthetic requirements during surgery. This effect is observed universally, regardless of the type of anaesthetic, i.v., volatile or regional block. For example, Ghingnone and colleagues [61] reported that premedication with oral clonidine 5 µg kg⁻¹, reduced fentanyl requirements for induction and intubation by 45 % in patients undergoing aortocoronary bypass surgery. In a similar patient population, Flack and colleagues [57] demonstrated that clonidine reduced sufentanil requirement by 40 %. Engelman and co-workers [49] showed that preoperative clonidine 5 µg kg⁻¹ decreased the dose

TABLE II. *Perioperative application of α_2 adrenergic agonists*

Drug	Route (dose)	Effect	Reference
Clonidine	Transdermal patch	Decreased isoflurane Haemodynamic stability	[131]
Clonidine	I.v. ($7 \mu\text{g kg}^{-1}$)	Postoperative analgesia Postoperative decreases in catecholamines and vasopressin Haemodynamic stability Less postoperative shivering	[116]
Clonidine	I.v. ($4 \mu\text{g kg}^{-1} + 2 \mu\text{g kg}^{-1} \text{h}^{-1}$)	Potential of morphine analgesia	[38]
Clonidine	Intrathecal (150 μg)	Prolonged bupivacaine spinal anaesthesia	[118]
Clonidine	Intrathecal (75, 150 μg)	Prolonged tetracaine spinal anaesthesia	[19]
Clonidine	Extradural (150 μg)	Potentiated lignocaine extradural anaesthesia	[146]
Clonidine	Extradural (90, 180 μg)	Sedation Haemodynamic stability	[99]
Clonidine	I.v. ($5 \mu\text{g kg}^{-1}$)	Decreased oxygen consumption	[117]

of droperidol necessary to maintain haemodynamic stability in aortic surgery patients. Doses of thiopentone and propofol required for induction are also reported to be reduced by preanaesthetic treatment by clonidine and dexmedetomidine [1, 3, 4, 104, 121]. This characteristic may facilitate more rapid emergence from anaesthesia. In addition, oral clonidine 150 μg may prolong tetracaine spinal anaesthesia [106].

α_2 agonists have been known to attenuate stress-induced sympathoadrenal responses. Minimizing haemodynamic changes after tracheal intubation and the noxious stimuli from surgery is an important goal for the anaesthetic care of the surgical patient. Carabine, Wright and Moore [28] suggested that clonidine 200 μg was a suitable dose and that larger doses did not offer any further advantage. Others have recommended larger doses of clonidine [60, 85, 153]. The efficacy of dexmedetomidine has been studied extensively in Finland. I.v. administration of the drug, in doses ranging from 0.3 to 0.6 $\mu\text{g kg}^{-1}$, provided optimal premedication effects [3, 4, 7, 75, 128]. Aantaa and co-workers [1, 2] studied i.m. dexmedetomidine, a clinically more appropriate route of administration, and showed that a dose of 1.0 $\mu\text{g kg}^{-1}$ was adequate. However, the sedative effects may outlast the surgery at these doses. Flacke and colleagues [57] reported that the haemodynamic variables in patients undergoing aortocoronary bypass surgery were more favourable despite the fact that smaller doses of narcotics were used. Ghingnone and co-workers [61] corroborated these findings in a similar patient population. While these features also applied to patients undergoing aortic surgery [49, 115], this favourable property was absent in patients undergoing carotid artery surgery [112]. The successful application of α_2 agonists to geriatric patients is established [60, 85], but this therapy has not been extended to a paediatric population.

Addressing possible disadvantages of α_2 agonists as premedication, oral clonidine 300 μg did not affect tidal volume, ventilatory frequency or end-tidal carbon dioxide tension, but did attenuate the ventilatory response to carbon dioxide, suggesting that clonidine has a potential respiratory depressant effect [12]. On the other hand, Bailey and colleagues [11] demonstrated that oral clonidine 4–5 $\mu\text{g kg}^{-1}$ did

not depress the response to carbon dioxide. Similar findings have been reported by Jarvis and co-workers also [77]. Furthermore, these two reports demonstrated that oral clonidine did not potentiate respiratory depression exerted by opioids. Another common risk with the use of α_2 agonists is excessive cardiovascular depression, namely bradycardia and hypotension. These complications were described in several reports [1, 2, 28]. Atropine is the treatment of choice for bradycardia, but it should be noted that large doses of oral clonidine, 5 $\mu\text{g kg}^{-1}$, attenuate the effect of atropine [100]. On the other hand, clonidine potentiates the pressor effect exerted by ephedrine [101].

Intraoperative administration (table II). Although α_2 agonists have potent analgesic and sedative effects, these agents have not been used during operation in lieu of the usual anaesthetic agents. Thus far, there are only a few reports describing intraoperative administration of α_2 agonists. Segal and co-workers [131] examined the efficacy of systemic clonidine. They reported that the combination of oral and transdermal clonidine (which maintained the plasma concentration of clonidine at therapeutic concentrations) provided smaller anaesthetic requirements, greater haemodynamic stability, more rapid recovery from anaesthesia and less postoperative requirement for supplementary morphine for pain control in patients undergoing lower abdominal surgery. Quintin and colleagues [116] reported that in patients undergoing abdominal aortic grafting, perioperative infusion of clonidine 7 $\mu\text{g kg}^{-1}$ over 120 min after aortic declamping, reduced noradrenaline, adrenaline and vasopressin concentrations during the recovery period. They concluded that although there was greater haemodynamic stability, larger fluid volumes were required during the postoperative period. Also, intraoperative clonidine infusion (a loading dose of 4 $\mu\text{g kg}^{-1}$ following 2 $\mu\text{g kg}^{-1} \text{h}^{-1}$ until closure of the peritoneum) may enhance the quality of postoperative analgesia by morphine [38]. A significant reduction in postoperative shivering episodes by clonidine treatment is also a consequence of this regimen.

α_2 agonists may also be administered by the intrathecal or extradural route to potentiate local anaesthetic agents. Racle and colleagues [118] showed that intrathecal clonidine 150 μg prolonged

TABLE III. Postoperative application of alpha₂ adrenergic agonists

Drug	Route (dose)	Effect	Reference
Clonidine	Extradural (2 µg kg ⁻¹)	Postoperative analgesia	[17]
Clonidine	Extradural (150 µg)	Postoperative analgesia	[53]
Clonidine	Extradural (100–900 µg)	Postoperative analgesia	[46]
		Haemodynamic depression	
		Sedation	
Clonidine	Extradural (400 µg + 20 µg h ⁻¹)	Less postoperative morphine	[96]
		Sedation	
		Prolonged local anaesthetics	
Clonidine	Extradural (150 µg + 50 µg h ⁻¹)	Postoperative analgesia	[27]
Clonidine	Extradural (150 µg)	Prolonged extradural fentanyl	[123]
Clonidine	Extradural (150 µg)	Prolonged extradural bupivacaine	[25]
Clonidine	Extradural (150 µg)	Potentiated extradural morphine	[27]
Clonidine	Intrathecal (150 µg)	Postoperative analgesia	[56]
Clonidine	I.m. (2 µg kg ⁻¹)	Postoperative analgesia	[17]
Clonidine	I.v. (150 µg)	Postoperative analgesia	[145]
Clonidine	I.v. (5 µg kg ⁻¹ + 0.3 µg kg ⁻¹ h ⁻¹)	Postoperative analgesia	[13]
		Haemodynamic depression	
Clonidine	I.v. (2 µg kg ⁻¹)	Decreased oxygen consumption	[39]
Dexmedetomidine	I.v. (0.2, 0.4 µg kg ⁻¹)	Less postoperative morphine	[6]
		Less postoperative shivering	
		Sedation	
		Haemodynamic depression	

bupivacaine spinal anaesthesia in elderly patients undergoing hip surgery and this technique was superior to the addition of adrenaline 200 µg to bupivacaine. Bonnet and co-workers [19] demonstrated that clonidine prolonged tetracaine spinal anaesthesia dose-dependently. Concerning extradural anaesthesia, addition of clonidine to extradural lignocaine was reported to increase its anaesthetic potency. Another advantage of extradural clonidine during surgery is the provision of sedation and relative haemodynamic stability compared with plain lignocaine or lignocaine with adrenaline [99].

Postoperative administration (table III). The potent analgesic properties of alpha₂ agonists provide improved pain control in postoperative patients. Extradural administration is the most common route and has been investigated thoroughly. The efficacy of clonidine after operation depends on the dose and the severity of postoperative pain. In an early clinical study, Gordh [63] failed to demonstrate a significant analgesic effect of extradural clonidine 3 µg kg⁻¹ in patients after thoracotomy. However, Bonnet and colleagues [18] observed that extradural clonidine 2 µg kg⁻¹ produced brief but significant pain relief after peripheral orthopaedic surgery. Recently, van Essen and co-workers [53] reported that extradural clonidine 150 µg produced postoperative analgesia in patients undergoing abdominal hysterectomy. On the other hand, Eisenach, Lysak and Viscomi [46] examined the analgesic effect of extradural clonidine 100–900 µg in 100-µg increments, in patients after total knee arthroplasty or abdominal surgery. Analgesia was estimated by verbal pain score and the need for supplementary i.v. morphine. Clonidine produced analgesia in a dose-dependent manner, achieving complete pain relief for up to 5 h without sensory or motor block at the largest dose (700–900 µg). However, larger doses were associated with disadvantages, including hypotension, bradycardia and transient sedation. Penon, Ecoffey and Cohen [109] reported that extradural clonidine 300 µg decreased the slope of

the ventilatory response to carbon dioxide without changing end-tidal carbon dioxide, ventilatory frequency or minute ventilation. To reduce the side effects without affecting the analgesic property, continuous extradural administration after bolus injection of clonidine (800 µg bolus followed by 20 µg h⁻¹) is advocated as an alternative method [96]. With this continuous treatment, more than 6 h of analgesia was seen after Caesarean section. In comparison with single dose clonidine administration, several reports describe the effectiveness of combining extradural administration of clonidine with local anaesthetics or opioids. Adding 150 µg of clonidine to extradural fentanyl, morphine and bupivacaine resulted in longer duration of postoperative analgesia [25, 27, 123]. Furthermore, these combinations may reduce the effective dose of clonidine, resulting in less side effects. In addition, a recent report described the efficacy of intrathecal clonidine 150 µg as the sole analgesic agent after Caesarean section without remarkable side effects [56].

Systemic administration of clonidine for postoperative analgesia has also been reported. Bonnet and colleagues [17] compared the analgesic effect (visual analogue scale) of i.m. clonidine 2 µg kg⁻¹ with the same dose of extradural clonidine after minor operations (orthopaedic or perineal surgery). Both onset and duration of analgesia after i.m. clonidine were comparable with extradural clonidine. In addition, although the peak plasma concentration of clonidine was greater in the i.m. group, side effects, including hypotension, bradycardia and drowsiness, were similar. Another possible route of systemic administration of alpha₂ agonists for postoperative analgesia is i.v. Tryba, Zenz and Strumpf [145] reported that clonidine 150 µg i.v., produced a similar analgesic effect to morphine 5 mg, in patients after orthopaedic operations. However, Striebel, Gottschalk and Kramer [139] could not demonstrate an analgesic effect of clonidine in patients after cholecystectomy. Aho and co-workers

[7] compared the analgesic effect of i.v. dexmedetomidine 0.2 and 0.4 $\mu\text{g kg}^{-1}$ with that of oxycodone 60 $\mu\text{g kg}^{-1}$ or diclofenac 250 $\mu\text{g kg}^{-1}$ in women undergoing laparoscopic tubal ligation. They showed that dexmedetomidine 0.4 $\mu\text{g kg}^{-1}$ produced a comparable effect to oxycodone, but drowsiness and bradycardia were present in the dexmedetomidine groups which, they suggested, may limit its usefulness. On the other hand, Bernard and colleagues [13] documented the efficacy of i.v. clonidine after major surgery. Their clonidine treatment regimen (5 $\mu\text{g kg}^{-1}$ infused in the first 1 h followed by 0.3 $\mu\text{g kg}^{-1} \text{ h}^{-1}$) was effective for pain relief in patients after extensive spinal surgery. They cautioned that adequate filling pressure must be maintained to prevent arterial pressure reduction.

Perioperative, as well as postoperative, administration of clonidine may decrease oxygen consumption and episodes of shivering during recovery from anaesthesia [39, 116, 117]. This feature provides further justification for using this agent in patients with coronary artery disease [57].

Miscellaneous usage. Because of their potent analgesic properties, α_2 agonists may be useful in the relief of pain other than in the postoperative period. Extradural clonidine produces effective analgesia in a dose-dependent fashion (100–900 μg) in patients with neuropathic pain with few side effects [48]. Extradural clonidine is a useful therapeutic adjunct in the management of patients with refractory reflex sympathetic dystrophy also [119]. Anecdotal reports demonstrate that intrathecal clonidine in combination with morphine or hydromorphone may attenuate cancer pain as well as opioids alone, and the combination was an excellent alternative approach to control of terminal pain [33, 52]. One case report suggested that intrathecal clonidine is effective, even if tolerance to intrathecal morphine has developed [34]. This phenomenon indicates the possibility that temporary pain control may be provided by clonidine to allow the morphine tolerant patient to recapture their sensitivity to morphine.

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OPIOID RECEPTORS AND THEIR RELEVANCE TO ANAESTHESIA

B. J. PLEUVRY

The therapeutic entities which have been derived from the constituents of opium, the dried exudate of the incised capsule of the opium poppy, have probably spawned more scientific publications than any other drug group. The observations that only the laevorotatory isomer of morphine, the active constituent of opium, was active and that most opioid analgesic drugs had similarities in structure, prompted Beckett and Casey to propose a tentative structure for an opioid receptor [4]. Since then our understanding of opioid pharmacology has progressed steadily with the notable highs of the discovery of the endogenous ligands to the opioid receptor [37] and the overwhelming evidence of multiple opioid receptors first noted by Martin and colleagues [56]. The anaesthetist uses morphine and its derivatives most commonly as analgesics for moderate to severe pain. With adjustment of dose and route of administration, opioids may perform this function with the minimum of side effects, which may range from the uncomfortable (itching, nausea and vomiting) to the life-threatening (respiratory depression). Chronic use of opioids may also lead to tolerance and physical dependence. It is worth re-emphasizing the theme which runs through most recent reviews of any aspect of opioid activity: "It was hoped that the development of opioid agonists selective for a particular opioid receptor subtype might separate the useful actions of opioids from the rest, and result in a therapeutic breakthrough. This breakthrough has not occurred!" What then, is the relevance of the subdivisions of opioid receptors to the anaesthetist?

OPIOID RECEPTORS: EVIDENCE FROM SELECTIVE LIGANDS

The different profiles of activities of morphine, ketocyclazocine and N-allyl-norcyclazocine (SKF 10,047) in the chronic spinal dog prompted the first well founded suggestion that subdivisions of opioid receptors might exist [56]. The first two (μ for morphine and κ for ketocyclazocine) mediate analgesia, which is generalized for the μ agonist but

spinal for the κ agonist. No cross tolerance exists between them. The third, σ for SKF, did not mediate analgesia and has subsequently been associated with the psychotomimetic activity of opioids. The next receptor to be described was the δ receptor [54] for which the endogenous opioid peptide leu-enkephalin showed selectivity. Originally this receptor was not associated with analgesic activity, but as we shall see later, this is not the case. The final receptor to be described (ϵ) is still an enigma. In a few, very specific situations, β endorphin appears to have agonist activity at opioid receptors which is not shared with morphine but which is antagonized by κ agonists [90]. Whether or not this represents a unique receptor (ϵ) or a lack of spare μ receptors at these β endorphin sensitive sites [61] is open to debate.

Further subdivisions of opioid receptors have been suggested based on selective ligand binding (table I).

μ receptors

The subdivision of μ receptors into μ_1 and μ_2 is based on the detection of two affinity states for the receptor [67] and on the selectivity of a single antagonist, naloxonazine [29], for the higher affinity state (μ_1). Meptazinol was claimed to be a selective μ_1 agonist but the intrinsic cholinomimetic activity of this compound clouded its pharmacology [5]. In contrast, morphine has greater selectivity for the low affinity site (μ_2), while the opioid peptides and morphine have similar affinities for the μ_1 site [39]. Published articles are polarized into those who do not accept a subdivision of μ receptors and, therefore, do not mention them, and those who treat their existence as an established fact.

δ receptors

Several lines of evidence support a subdivision of δ receptors. Two highly selective δ agonists have been described. Both the synthetic peptide [D-Pen², D-Pen⁵] enkephalin [62] and [D-Ala²] deltorphin II, isolated from frog skin [47], bind with high affinity to δ receptors and exhibit analgesic activity in animal models [41]. This analgesic activity is antagonized

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TABLE I. *Selective ligands for opioid receptors. †No selectivity for receptor subtypes; ‡agonist/antagonist status is controversial [85]. ICI 174,864 = N,N-diallyl-L-tryptophan- α -aminoisobutyric acid- α -aminoisobutyric acid-Phe Leu OH*

Receptor	Subtype	Agonist	Antagonist
μ	μ_1	Meptazinol Morphine = opioid peptides	Naloxonazine
	μ_2	Morphine > opioid peptides	β -Funaltrexamine†, cypridime†
δ	δ_1	[D-Pen ² D-Pen ⁵] enkephalin	[D-Ala ² Leu ⁵ Cys ⁶] enkephalin (DALCE),
	δ_2	[D-Ala ²] deltorpin II	Naltrindole-5'- isothiocyanate (5NTII) Naltrindole†, ICI 174,864†
κ	κ_1	U69,593	Nor-binaltorphimine
σ	κ_2 (ε?)	NalBzoH	
	κ_3	Phencyclidine > benzomorphans	
	PCP	(+) Pentazocine, benzomorphans† > phencyclidine†, haloperidol†‡, (-) pentazocine†	
	σ_1		
	σ_2		

by the selective δ antagonist ICI 174,864, but not by the selective μ antagonist, β -funaltrexamine. However, there is no cross tolerance between the analgesic activity of these two compounds in mice [57], suggesting that they do not interact with identical receptors. Furthermore, each drug may be selectively antagonized by one of two novel δ receptor antagonists (table I) [42]. Additionally, these two agonists appear to activate different signal transduction mechanisms (see below). There is some evidence [32] that μ and δ receptors may exist in two forms, complexed together and non-complexed. If this is true, it could explain some of the observations regarding subdivisions of μ and δ receptors discussed above. The functionally coupled μ - δ receptor is sensitive to β -funaltrexamine, but not naloxonazine, which is believed to be selective for μ_1 receptors.

κ receptors

Binding selectivity studies have produced evidence of three subdivisions of κ receptors. Both κ_1 and κ_3 receptors appear to have selective agonists, (5a,7a,8b)-(-)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)-de-8-yl] benzenacetamide (U69,593) for κ_1 receptors [92] and naloxone benzoylhydrazone (NalBzoH) for κ_3 receptors [9, 11]. The highly selective κ receptor antagonist nor-binaltorphimine [82], has poor affinity for the κ_3 site. It has been suggested that the first drug used to antagonize morphine-induced respiratory depression, nalorphine, may exert its intrinsic analgesic activity via this κ_3 site [69]. The κ_2 site is characterized more by what does not bind to it rather than what does (i.e. it is insensitive to U69,593 and NalBzoH). Nock and co-workers [63] also described a U69,593 and NalBzoH insensitive binding site for the κ agonist ethylketocyclazocine. It was concluded that this might be the β endorphin specific receptor, the ϵ receptor! Perhaps κ_2 and ϵ are the same receptor! However, there are still no selective ligands for this binding site as β endorphin binds to μ , δ , κ_3 and κ_1/ϵ sites [9].

σ receptors

The original σ receptor ligand SKF 10,047, was found to bind to both the PCP (1-(1-phenylcyclohexyl)piperidine, commonly known as phencyclidine or "angel dust") receptor and the σ receptor. The rank order of potency for binding to these two receptors differs: benzomorphans have greater affinity than PCP at the σ site and vice versa at the PCP site [74]. The PCP receptor is now known to be present in the ion channel gated by the N-methyl-D-aspartate receptor [52] whilst the σ receptor has been proposed to have two subtypes, σ_1 and σ_2 [73]. σ ligands, such as (+)-pentazocine, dextromethorphan and SKF 10,047, have much greater affinity for σ_1 than σ_2 receptors. (-)-Pentazocine and many antipsychotic drugs are non-selective ligands at σ receptors and naloxone is inactive [85]. For this last reason, σ receptors are no longer classified as opioid receptors.

SIGNAL TRANSDUCTION MECHANISMS

Several of the opioid receptors have been solubilized and purified, but details of their primary structure, at least of those that may mediate analgesic activity, await elucidation [53]. It is hoped that this information will be available by the time this review is published. For the present, only indirect pointers to the nature of the opioid receptors may be used. Indicators predict that most of the opioid receptors characterized so far are members of the guanine nucleotide binding protein (G protein)-coupled receptor super family. Guanine nucleotides, particularly guanosine triphosphate (GTP), decrease specifically agonist binding to the μ , δ and κ opioid receptors and opioid agonists stimulate GTPase activity. In addition, in the presence of sodium, guanine nucleotides discriminate effectively between agonist and antagonist binding: the antagonist binding is unaffected by GTP concentrations. These findings are characteristic of G protein-coupled

receptors. GTP sensitivity is also seen with σ_1 receptors, but not with σ_2 receptors [73]. G proteins are heterotrimers, that is consisting of three different subunits, designated α , β and γ in order of decreasing molecular weight. The α subunit binds the guanine nucleotides and differences in this subunit are used to identify different G proteins. The G_s notation was used originally to designate a G protein which stimulated (s) adenylyl cyclase and G_i , a G protein which inhibited (i) this enzyme. Eventually this method of classification will change as more G proteins are identified and the heterogeneous character of the presently described G proteins is elucidated (e.g. G_i has at least three forms).

Two toxins, cholera and pertussis, are known to catalyse covalent modifications (adenosine diphosphate (ADP) ribosylation) of the α subunit. Cholera toxin has selectivity for the α subunit of the stimulatory G_s protein, causing persistent activation [25]. This induces persistent activation of adenylyl cyclase in, for example, the intestinal epithelium, which leads to copious intestinal secretions, characteristic of the disease [75]. Pertussis toxin, on the other hand, causes ribosylation of G_i and other G proteins, but not G_s proteins. Pertussis toxin modifies the ability of the G protein to bind to the receptor and thus prevents its activity [25]. How this causes the symptoms of pertussis is less clearcut and may reflect the heterogeneity of G_i proteins alluded to above.

In some, but not all systems, opioid agonists have been shown to inhibit adenylyl cyclase. This effect is also GTP sensitive and is blocked by pertussis toxin. These findings are consistent throughout the literature and demonstrate that opioid receptors (the evidence is greater for μ and δ than κ) inhibit adenylyl cyclase via a G_i protein [10]. Pertussis toxin reduces morphine-induced analgesia when injected intraventricularly [66] and intrathecally [34], thus implicating G_i , or at least a pertussis sensitive G protein, in this action. Similarly, G_i protein activation has been implicated in the place preference properties of morphine [80].

Cholera toxin, which activates G_s , also prevents the analgesic action of morphine, indicating the complex circuitry involved in pain and analgesia [7]. There is some evidence that direct activation of G_s proteins may be involved in the excitatory actions of opioids [13]. Uncovering of these excitatory effects by pertussis toxin has been offered as an explanation of the observation that, while pertussis toxin attenuates opioid-induced analgesia, it enhances lethality [55]. However, no direct evidence was offered as to the cause of death in these animals, although naloxone was preventative, suggesting involvement of opioid receptors.

Whether or not adenylyl cyclase is implicated as a second messenger in all opioid activity is debatable. G proteins are, as quoted so descriptively by many reviewers, promiscuous. They may interact directly with ion channels, as well as with several other second messenger systems, such as guanylyl cyclase, phospholipase C-inositol phosphate, phospholipase A2, etc. The involvement of some of these other systems with opioid activity is suggested from a

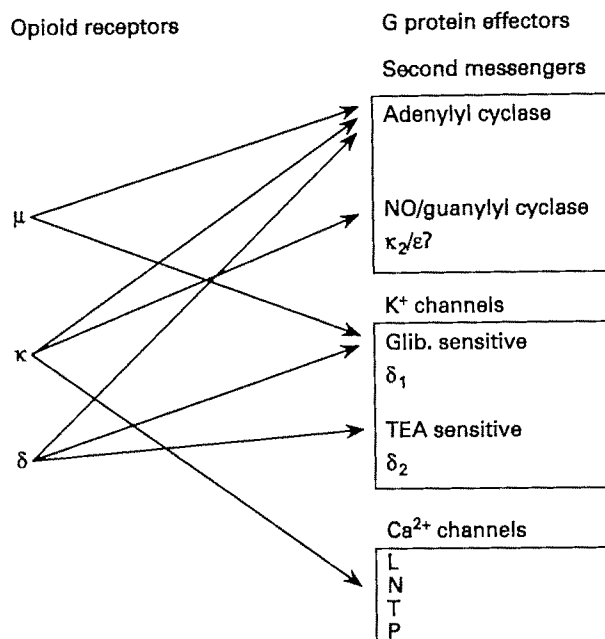


FIG. 1. Associations between opioid receptors and G protein effectors. Glib. = Glibenclamide.

number of sources. In the locus coeruleus, the response to agonist- μ receptor interaction is activation of potassium conductance via a G protein with no other detectable second messenger system involved [46]. Both μ and δ receptors appear to be associated with the opening of potassium channels, whilst κ receptors are associated with the closure of calcium channels [14], at least when inhibitory activity is recorded. To date, potassium channels are the most diverse group of ion channels investigated. At least 13 major types exist and several subtypes exist within each group [20]. Glibenclamide, believed to be a selective blocker for the ATP sensitive potassium channels in the CNS [2], antagonizes morphine-induced analgesia, whilst tetraethylammonium and quinine, which block other types of potassium channel [12], have no effect [64]. The potassium channel opener pinacidil, potentiates morphine analgesia in the rat [84]. Using ligands selective for the proposed δ_1 and δ_2 receptors discussed above, Wild and colleagues [89] found that δ_1 -induced analgesia was antagonized by glibenclamide and not tetraethylammonium (TEA) whilst the δ_2 agonist was antagonized by TEA and not glibenclamide. It would be logical that, if some μ and δ receptors are complexed (as outlined above), they should be the ones linked to the same effector system (i.e. the glibenclamide-sensitive potassium channel). Agonists at κ receptors, which are believed to act through calcium channels, were unaffected by either of the potassium channel blocking drugs discussed above. Four types of calcium channels have been described, L, N, T and P. The L channel, which is blocked by calcium antagonists, such as nifedipine, is particularly important in smooth muscle and cardiac muscle contraction and is located in some neurones. However, it is the N channels, which have been identified only in neurones and appear to participate in neurotransmitter release, which are selectively inhibited by the κ agonist, dynorphin A

[28]. Only a proportion of N channels are sensitive to κ agonists.

Nitric oxide is synthesized by certain mammalian cells, including brain cells, as a biological messenger molecule which activates guanylyl cyclase. The role of this in the central nervous system is unknown, but addition of the nitric oxide precursor L-arginine, potentiates β endorphin-induced analgesia (or antinociception), whilst N ω -nitro-L-arginine, an inhibitor of nitric oxide production, prevents β endorphin-induced analgesia [83]. There is also some suggestion that a nitric oxide-cyclic GMP signalling pathway is involved in peripheral analgesia [18], but whether or not this is important for the peripheral activity of opioids (discussed below) is unclear (fig. 1).

It is particularly ironic that the only opioid binding protein which has been cloned and sequenced at the time of writing is not a G protein-coupled receptor. Its amino acid sequence places it in the immunoglobulin super family, which includes the interleukin-6 receptor [53]. This binding protein may be relevant to the activities of opioid agonists on the immune response, which has been reviewed by Sibinga and Goldstein [76].

FUNCTIONAL RELEVANCE OF OPIOID RECEPTORS

As mentioned above, the most important pharmacological property of opioid drugs is analgesic activity. It is now apparent that opioids exert this effect supraspinally, at the level of the brain stem, spinally by inhibiting nociceptive input, and peripherally when pain is produced by inflammation or a sympathetic pain syndrome.

Supraspinal injections of μ , κ and δ agonists produce naloxone-sensitive analgesia. However, in most instances, activation of μ receptors may explain the analgesic activity observed [14]. However, there is evidence that analgesia occurs through activation of δ receptors [33]. The independent supraspinal analgesic activity of κ receptor activation is uncertain and all shades of opinion may be found in the literature [14, 49, 60]. While the role of μ receptors in supraspinal analgesia is accepted unequivocally by most authors, the exact mechanism by which this effect is achieved is exceedingly murky. There is contradictory evidence that morphine both enhances and inhibits descending inhibitory control of spinal nociceptive pathways (e.g. [1, 15, 24, 26]). Authors on both sides of the divide have produced interesting hypotheses as to how their findings might explain observations concerning the analgesic action of morphine. However, few address the problem as to why these studies produce opposing views.

The observation that opioids cause analgesia at a spinal site has led to increasing use of extradural or spinal administration of opioids for pain relief [22]. The maximum pain relief achieved by this route is less than that achieved when there is some spread of the drug to supraspinal sites, although this spread is accompanied by an inevitable increase in side effects, such as respiratory depression. Some drugs, such as pethidine and fentanyl, possess additional local

anaesthetic activity [71] and this may increase their ability to produce pain relief via the spinal or extradural route [27]. It is most likely that the spinal analgesic activity of opioids is mediated via μ receptors, as these make up the largest proportion of receptors in the spinal cord. These receptors are found close to the C-fibre terminals in lamina I and in the substantia gelatinosa. In addition, all clinically used opioids have significant μ receptor activity. Naloxonazine does not antagonize the analgesic activity of μ agonists in the spinal cord, although it does antagonize supraspinal μ -induced analgesia. This could indicate that μ_2 receptors mediate spinal analgesia, whilst supraspinal analgesia is μ_1 -mediated [68]. However, both μ and δ receptor selective agonists inhibit C-fibre activity: this effect is sensitive to selective receptor antagonists [70]. Very small doses of μ agonists actually enhance C-fibre activity: this effect is not shared by δ agonists [16]. This may be a result of activation of G_s proteins (see above). It has been suggested that this enhancement of C-fibre activity may account for the itching seen after spinal opioid injections in man [14].

Synergy between the spinal antinociceptive actions of μ and δ agonists has been reported by several groups [16, 59, 65]. Thus enhanced analgesic efficacy of spinal opioids may be obtained by combining drugs with μ and δ receptor activity. As with the supraspinal site, the role of κ receptor activation in the production of analgesia is controversial. While drugs with κ agonist activity produce analgesia at the spinal level, many possess activity at other receptors, both opioid and non-opioid, which could explain their action. For example, trans-3,4-dichloro-N-methyl-N-[(2 pyrrolidiny)-cyclohexyl] benzene acetamide (U50,488H) has been used as a selective κ agonist, but it has been shown to have non-opioid analgesic effects *in vivo* [31]. In addition, the κ antagonist, nor-binaltorphimine, which shows good selectivity for κ receptors *in vitro*, requires large doses and prolonged exposure to be effective *in vivo* and may not discriminate between μ and κ receptors [6]. Some studies have even shown an anti-analgesic action of dynorphin A, a proposed endogenous ligand at κ receptors [23].

Powerful analgesic effects in inflammatory pain may be obtained with opioids acting at peripheral receptors. Early experiments failed to demonstrate unequivocally that these receptors were truly opioid in nature, as antagonist reversibility was questionable [21]. Subsequently, evidence was produced demonstrating that these peripheral effects were stereospecific and naloxone sensitive, suggesting true opioid receptors. The use of selective agonists and antagonists also suggested that these receptors had characteristics resembling μ , δ and κ receptors [78]. Inflammatory mediators, such as prostaglandin E_2 (PGE $_2$), activate adenylyl cyclase via a stimulatory G protein causing nociceptor sensitization. Activation of μ receptors switch off this process via G_i and thus prevent nociceptor sensitization [50]. Prevention of PGE $_2$ hyperalgesia is not a property shared by δ and κ agonists, but they can prevent bradykinin-induced hyperalgesia. Bradykinin induces the release of nociceptor sensitizing agents, including the pros-

tanoids, from postganglionic sympathetic nerves, thus it is thought that δ and κ receptors mediating peripheral analgesia are situated on the sympathetic nerves and prevent the release of noxious mediators [81]. These interesting observations prompted a study which resulted in the successful use of morphine injected intra-articularly for pain relief after arthroscopic knee surgery [77], and may mean the more extensive use of peripherally applied opioids [3]. Indeed reports on the benefits of intra-articular morphine are beginning to appear in the anaesthetic literature [44].

Although a significant proportion of the discussion above has concentrated on the possible roles of δ and κ receptors in opioid-induced analgesia, it must be emphasized that the main analgesic component in the activity of presently available opioids is mediated via the μ receptor.

INVOLVEMENT OF OTHER NEUROTRANSMITTER SYSTEMS

Pain pathways and their modulation involve many neurotransmitters in addition to the endogenous opioids. In the recent past, particular attention has been focused on the roles of the excitatory amino acids, noradrenaline and cholecystokinin, in pain pathways and in modulation of opioid analgesic activity (table II).

Amino acids

The excitatory amino acids, glutamate and aspartate, combine with at least five subtypes of receptor, known as N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) (previously quisqualate), kainate, L-2-amino-4-phosphonobutyrate (L-AP4) and metabotropic [87]. These receptors are named after selective agonists. The NMDA receptor has been characterized the best and is known to be part of a ligand-gated ion channel.

Both glutamate and NMDA produce analgesia when injected into the periaqueductal gray matter and this effect is not naloxone sensitive. However, the competitive NMDA antagonist, (–)-2-amino-7-phosphonoheptanoate ((–) AP-7), antagonized the actions of both morphine and NMDA at this site [40]. It was concluded that morphine disinhibited neurones containing NMDA receptors. Systemic administration of the non-competitive NMDA receptor channel complex antagonist, MK 801 (dizocilpine), also reduced morphine-induced analgesia [51]. In the nucleus raphae magnus, long lasting opioid-induced analgesia was not inhibited by NMDA antagonists, but was reduced by a

selective quisqualate/kainate receptor antagonist, indicating the involvement of non-NMDA receptors at this site [72]. In contrast, NMDA receptors have also been implicated in increases in nociceptive responsiveness, that is amplification of noxious inputs. Prior administration of opioids prevents this hyper-responsiveness [14]. It would appear that the co-administration of opioids and NMDA antagonists may be useful or harmful, depending on the characteristics of the pain being treated! Inhibitory amino acids may also have a role in opioid analgesia as intrathecal midazolam potentiates morphine in doses which are without effect when injected alone [91]. In addition, there are clear links between opioid receptors and GABA_A disinhibition of interneurons [19].

Monoamines

Over the past few years, there has been increasing interest in the potential use in anaesthesia of drugs acting at α_2 adrenoceptors [58]. Agonists at α_2 receptors, such as dexmedetomidine, have both sedative and analgesic activity and reduce the dose requirements for i.v. and inhalation anaesthetics and opioid drugs. There is clear synergy between opioids and α_2 agonist analgesia and in some experimental situations, dexmedetomidine-induced analgesic activity has been antagonized by naloxone [79]. It is unlikely that this is a result of direct stimulation of opioid receptors by dexmedetomidine, but it does suggest close links between the two systems, at least in the spinal cord. In the locus coeruleus, both μ opioid receptors and α_2 adrenoceptors regulate the same potassium conductance [46]. Using tail pinch as a method of studying analgesia, evidence has been presented that κ opioid agonists, but not morphine, activate noradrenergic and serotonergic pathways in mice [48]. However, as the authors point out, the method of testing for analgesic activity has a significant influence on the results obtained in this type of experiment.

Histamine, via its H₂ receptor, has been suggested as a mediator of antinociceptive responses [35]. Direct administration of histamine H₂ antagonists into the brain had no effect on baseline nociceptive scores, but inhibited the action of morphine. The maximum inhibition was 70 to 80% with larger doses causing less effect (i.e. a U-shaped dose-response relationship) [36]. These findings, which were found using two separate tests of antinociceptive activity, were interpreted as evidence for the involvement of histamine in the antinociceptive activity of morphine.

Cholecystokinin

The cholecystokinin (CCK) group of peptides are distributed widely in the brain and periphery. The 33 amino acid form, CCK-33, is secreted from endocrine cells whilst neuronal tissue utilizes the octopeptide CCK-8. The A type cholecystokinin receptor is found in greater concentrations in the periphery than the B type receptor. Rodents have relatively few CCK-A receptors in the brain, but this is not true of primates [38]. There are a number of

TABLE II. Modification of morphine analgesia by opioid and non-opioid transmitter systems (see text for details)

Analgesia reduced	Analgesia enhanced
Dynorphin A (κ opioid agonist)	δ opioid agonists
NMDA antagonists	GABA _A agonists or enhancers
Histamine H ₂ antagonists	α_2 adrenoceptor agonists
CCK agonists	CCK-B antagonists

suggestions in the literature that cholecystokinin may act as an antagonist system to the endogenous opioids. This appears to be specific for the μ or κ opioid system as analgesia elicited by δ opioid receptors [86] or non-opioid mechanisms are not affected [30]. Acutely, cholecystokinin antagonists and antibodies enhance morphine-induced analgesia, but this effect is lost on chronic treatment [45]. CCK-B receptors are believed to mediate opioid interactions [17], although this may reflect the relative lack of CCK-A receptors in rodent brain. It has been suggested that endogenous opioid systems are engaged when danger is signalled, so that pain can be ignored and the chances of survival are increased. In contrast, safety signals could switch on cholecystokinin systems so that any wounds would be attended to promptly [88]. Using this reasoning, Wiertelak, Maier and Watkins [88] suggested that rapid tolerance to opioids may be expected when given in a familiar non-stressful environment. In contrast, the fear-provoking environment of hospitals may be expected to retard tolerance development.

The cholecystokinin story is complicated by earlier reports in the literature that CCK-8, in the absence of opioid activity, could produce analgesia when injected systemically and centrally [43].

In view of the above discussion, it is perhaps not surprising that the opioid antagonist naloxone should exhibit both analgesic and antanalgesic properties, depending on the circumstances [8]. The nociceptive state depends on a balance between the activity of all opioid peptide systems interacting with its various receptors, as well as other neurotransmitters acting via opioid and non-opioid systems. As our understanding of the mechanisms of pain and analgesia increase, one may only be thankful that man found opium by serendipity and used it as an analgesic, before trying to discover the mechanisms behind pain and analgesia.

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GABA RECEPTORS AND BENZODIAZEPINES

C. S. GOODCHILD

From an historical standpoint, the development of our understanding of basic mechanisms of the actions of gamma aminobutyric acid (GABA) and benzodiazepines began on separate paths. Classical pharmacology revealed similarity between the actions of GABA and benzodiazepines in the central nervous system. Furthermore, pharmacological experiments with selective agonists and antagonists indicated that there exists subtypes of both GABA and benzodiazepine receptors and that the actions of the benzodiazepines are produced by modulation of GABA effects. Thus the two paths of research on GABA and benzodiazepines had crossed in explaining the effects of benzodiazepines by an interaction with GABA_A receptors that were located on the same macromolecular complex. More recently, this interaction has been elucidated further by the cloning of subunits of GABA_A receptors: the interaction between GABA and benzodiazepines is now being explained at a molecular level.

GABA

Hayashi first proposed GABA as an inhibitory neurotransmitter in the mammalian central nervous system in 1954. This was based on the observed presence of the amino acid in the brain and the anticonvulsant properties of the compound when injected into animals. Supporting evidence for this concept came when GABA was extracted from the central nervous system, identified chemically and shown to have inhibitory actions on the nervous system [3, 25]. In mammals, GABA is present almost exclusively in the central nervous system, although small amounts may be found in other areas such as autonomic ganglia. It is a simple amino acid molecule which is formed by decarboxylation of L-glutamic acid by the enzyme glutamic acid decarboxylase (GAD). This enzyme is found only in the cytoplasm of neurones containing GABA.

Although distributed widely in the central nervous system, the concentration of GABA varies in different regions with the greatest concentrations found in basal ganglia, hippocampus, cerebellum and hypothalamus in the brain, and in the substantia gelatinosa of the dorsal horn of the spinal cord.

Large concentrations are found also in the cerebellum and retina. GABA in the retina is localized mostly in the horizontal cell layer where it is responsible for lateral inhibition.

Most GABAergic neurones are interneurons, with short axons and local connections. Generally the actions of GABA within the central nervous system are inhibitory. This effect is achieved by an increase in chloride conductance [4, 24, 30, 31]. Pre- and postsynaptic effects have been described. For example, GABA has long been known to produce presynaptic inhibition by hyperpolarization of afferent terminals in the spinal cord [16]. This effect is shown in figure 1. The GABAergic interneurone releases GABA onto a primary afferent fibre before it makes a synaptic contact. The effect of this is to cause hyperpolarization of the primary afferent fibre. This effect may be recorded from the cut central ends of these primary afferent fibres as a "dorsal root potential". The hyperpolarization decreases the central propagation of the action potential. Thus less excitatory primary afferent neurone transmitter is released onto the postsynaptic neurone.

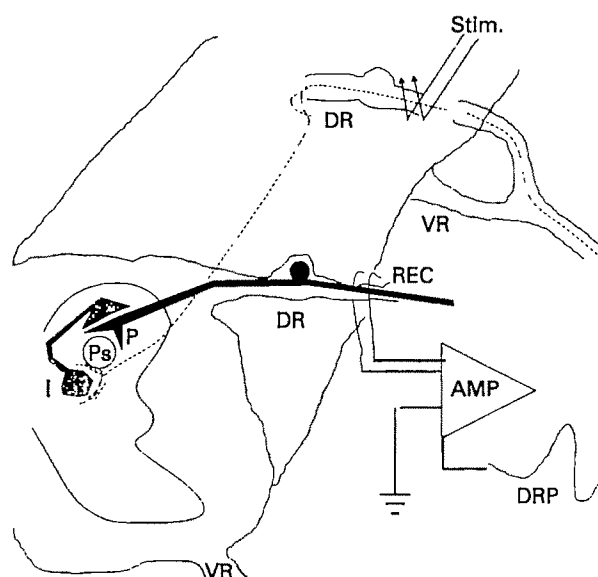


FIG. 1. GABAergic presynaptic inhibition. The shaded interneurone releases GABA onto the presynaptic terminal (P). DRP = Dorsal root potential; VR = ventral root; Ps = postsynaptic cell; Stim = stimulation; REC = recording electrodes.

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KEY WORDS

Hypnotics: benzodiazepines, diazepam, midazolam. Receptors: GABA.

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Pharmacological studies have indicated functions for GABA within the central nervous system in the induction of sleep, control of neuronal excitability/epilepsy, anxiety, memory and hypnosis. Evidence for the involvement of GABA in these widely varying functions comes from the effects of the compound itself when injected into animals, and also from the effects of drugs that either alter the synthesis or metabolism of GABA or act at postsynaptic GABA receptors. Thus glutamic acid decarboxylase inhibitors lead to reduction in brain concentrations of GABA. This ultimately leads to convulsions. Drugs which block the reuptake of GABA from the subsynaptic cleft back into the presynaptic terminal, such as diaminobutyric acid, lead to increased concentrations of GABA. Such drugs possess anti-convulsant and sedative properties. Bicuculline and picrotoxin, which bind to the postsynaptic GABA receptor, are convulsants in large doses and in smaller doses alter memory.

Hypnosis

The sedative and hypnotic effects of GABA were the main properties of interest to early researchers. Sedation was found to be of anaesthetic interest. For example, Galindo [26] showed that halothane potentiates the inhibitory action of GABA in the cuneate nucleus. These observations led to one of the theories of anaesthetic action, that is potentiation of central nervous system depression by GABA. Other anaesthetic drugs are known to interact with GABA in this respect: propofol [50], barbiturates [1, 23, 69] and neurosteroids [52].

Epilepsy

The role of GABA in the prevention of epilepsy is now well established. Many of the conventional drugs used in the management of epilepsy are known to interact with GABA receptors (*vide infra* for benzodiazepines, barbiturates and neurosteroids). Sodium valproate is thought to produce its anti-convulsant action by reducing the metabolism of GABA. More recently introduced drugs include GABA analogues such as vigabatrin [32].

Anxiety

Anti-anxiety effects of GABA in animals are reduction in aggression and increase in social behaviour, thus allowing the animals to be handled more easily. This action of GABA is thought to be a result of interaction with 5-hydroxytryptamine (5-HT) in the limbic system. These interactions are known to occur at a number of sites within the nervous system [48] and also explain the anxiolytic effects of 5-HT₃ receptor antagonists [14, 15]. It is interesting to note that GABA_A receptor subunit mRNA (*vide infra*) are increased by social stress in animals [35]. This observation indicates that this is a real physiological role for GABA.

Memory

The first evidence for involvement of GABA mechanisms in memory storage and retention came from the observation that systemic picrotoxin, which is an antagonist at the postsynaptic GABA receptor,

enhanced both these functions [9, 13, 41, 42]. Involvement of the GABA_B subtype receptor in the laying down of memory has also been indicated by the observation that microinjections of the GABA_B receptor agonist baclofen, impair memory [12].

Two subtypes of GABA receptors have been suggested by classical pharmacological experiments and these have been denoted GABA_A and GABA_B. They are characterized by different rank orders of potencies of available agonists for each of these two receptors and selective action of antagonists. GABA binds to both types of receptor. Baclofen is a potent GABA mimetic selective for the GABA_B receptor; it is inactive at the GABA_A receptor. Muscimol and isoguvacine are potent GABA mimetics selective for the GABA_A subtype. Although the GABA_A subtype is of primary interest to this review because this is responsible for benzodiazepine actions, GABA_B receptors will be considered briefly.

GABA_B receptors

GABA_B binding sites were described first in peripheral tissue preparations in which they were located presynaptically on autonomic nerve terminals [5]. However, further studies indicated a similar presynaptic location in central nervous system neurones, with functions being diminished release of amines, neuropeptides, hormones and excitatory amino acids, as well as GABA via autoreceptors (i.e. GABA_B receptor binding presynaptically on a GABAergic neurone leads to diminished release of gamma aminobutyric acid from that neurone) [68].

GABA_B and GABA_A binding sites may be demonstrated in the central nervous system by autoradiography. The distribution of the two receptor subtypes is quite different. GABA_B receptors are located both presynaptically on nerve terminals and postsynaptically in many brain regions, as well as in the dorsal horn of the spinal cord. In the spinal cord dorsal horn, 50 % of GABA_B binding sites have been reported to be associated with small diameter primary afferent fibres [56].

The functional role of GABA_B receptors in the central nervous system is still understood poorly. The wide distribution in the nervous system suggests they are likely to have many effects. One therapeutic benefit of GABA_B agonists is control of spasticity. Baclofen (GABA_B agonist) given orally or intrathecally, reduces spasticity and prevents flexor spasms in a number of different neurological disorders. This is thought to be a result of interruption of mono- and polysynaptic spinal reflexes [17].

GABA_A receptors

GABA_A receptors are stimulated by GABA, muscimol and isoguvacine. Molecular biological studies performed in the past decade indicate that the A subtype of GABA receptor may be a group of different receptors rather than a single entity. Four types of subunits have been described: α , β , γ and δ [2, 28, 75]. Combinations of these subunits produce macromolecular complexes which include a chloride ion channel. It has been noted that there are many similarities with the nicotinic acetylcholine receptor.

It has been proposed that the GABA_A receptor has a similar structure (i.e. a pentameric form). Combination of GABA and a GABA_A subtype receptor leads to an increase in chloride conductance and subsequent hyperpolarization of the postsynaptic membrane.

Multiple variants exist within each of these classes: six α , three β , two γ and one δ [36, 44, 58, 73]. Analysis of GABA receptors within the retina have yielded two other subunits, $\rho 1$ and $\rho 2$. Each of the subunits contains an N-terminal segment of approximately 200 amino acids and four hydrophobic membrane-spanning segments. Each of these four segments is connected to the others by short hydrophilic segments of amino acids, except for the third and fourth which have a longer segment that may be phosphorylated by intracellular cyclic AMP-dependant protein kinase (protein kinase A). This opens up the possibility for a large number of interactions with other drugs that may alter intracellular cyclic AMP levels, such as: opioids (μ and δ); purinoceptors; α_1 adrenoceptors (subtypes A and B); beta adrenoceptors; dopamine D1 and D2; GABA_B; 5-HT (subtypes 1A, 1B and 1D); muscarinic; cholinergic (M2); prostanoid DP receptors; and neurophysin and oxytocin receptors (subtype V₂) [47].

Co-expression studies using cloned GABA_A subunit variants in mammalian cells have led to combinations of subunit that respond to GABA, and this response may be modulated by benzodiazepines [43, 57]. The presence of a γ subunit confers benzodiazepine sensitivity [37]. Less well defined actions at GABA_A receptors have also been described for barbiturates and neurosteroids (e.g. alphaxalone, alphadolone, both constituents of Althesin, and pregnanolone, currently in phase I trials as an i.v. anaesthetic) [11, 21, 33, 55, 59, 74]. Although it is possible to make a number of different combinations of these subunits, apart from the alteration in benzodiazepine specificity and sensitivity (*vide infra*), the functional significance of this diversity has yet to be elucidated [49].

BENZODIAZEPINES

The first clinically useful benzodiazepine, chlor-diazepoxide hydrochloride, was discovered in 1957 after a search for compounds with sedative and anxiolytic properties related to 4,5-benzo-hept-1,2,6-oxdiazines, compounds known since 1891 [66]. These chemicals lend themselves readily to modification by organic chemists so that a large number of related compounds were synthesized. Three basic types of structure exist: basic 1-4 benzodiazepines, such as diazepam and lorazepam (see fig. 2); heterocyclic 1-4 benzodiazepines, such as triazolam (fig. 2); 1-5 benzodiazepines, such as clobazam (fig. 2).

All of these compounds possess similar properties, that is reduction of anxiety, sedation and anti-convulsant properties. However, the 1-5 benzodiazepines have been found to be less sedative. These drugs also have significant muscle relaxant properties, without any effect on coordination. This

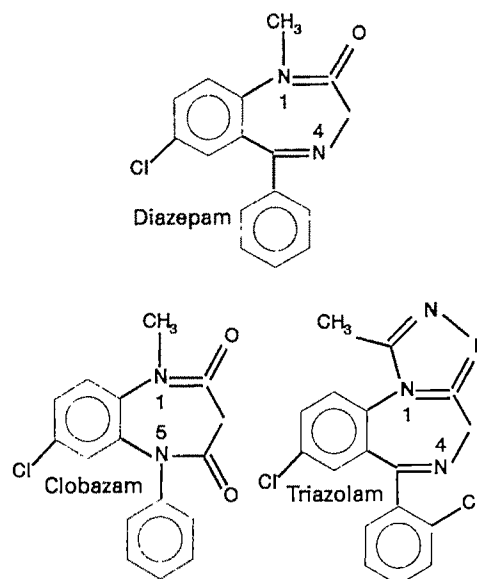


FIG. 2. Molecular structure of three benzodiazepines.

latter effect may be because of an antianxiety action, but is probably mediated also by potentiation of the effects of GABA on spinal and supraspinal motor reflexes. As the dose of benzodiazepine is increased, anxiolytic effects are produced first followed by anticonvulsant effects and then a reduction in muscle tone, followed by sedation and hypnosis. The similarity between these effects and those of GABA was obvious. It was logical to look to GABA to explain the therapeutic effects of this group of compounds.

GABA-BENZODIAZEPINE INTERACTION

Before Davidoff's suggestion of a role for GABA in mediation of presynaptic inhibition [16], Schmidt, Vogel and Zimmerman [62] showed that diazepam increased the amplitude of the dorsal root potential, and enhanced monosynaptic ventral root responses. Polc and colleagues [53] investigated this further and showed an interaction between diazepam and GABA. Benzodiazepines alone lack GABA mimetic effects. However, they have been shown to enhance the effects of GABA in numerous regions in the central nervous system, for example spinal cord, cerebellum, hippocampus, cuneate nucleus, cerebral cortex, hypothalamus, amygdala and brain stem raphe nuclei [34, 69].

Benzodiazepine receptors

Specific high affinity benzodiazepine binding sites were demonstrated in the central nervous system in 1977 [7, 46]. These specific binding sites are located on neurones within the central nervous system, but also on non-neuronal tissues, perhaps glia. The function of these is unknown [20]. Binding studies with agonists and antagonists promoted the concept of these recognition sites as functional receptors. Drugs acting stereospecifically with this receptor may be classified as selective agonists (e.g. flunitrazepam) that potentiate GABA effects, inverse agonists, such as DMCM or Ro194603 that produce exactly opposite effects (proconvulsant) and an-

tagonists, such as flumazenil, that stereospecifically block the effects of either agonists or inverse agonists. A strong correlation between binding and pharmacological activity has been reported.

Haefely suggested that a GABA_A receptor, benzodiazepine receptor and chloride ionophore formed one macromolecular complex in order to explain experimental observations that benzodiazepines were not GABA mimetic *per se*, but potentiated the effects of binding with GABA_A receptors in operating a chloride channel [24, 29, 63]. Not all GABA receptors are coupled to benzodiazepine receptors; this macromolecular complex forms a subset of GABA_A receptors. This subspecialization of GABA_A receptors with respect to the benzodiazepines goes further. The first indications of this came from the pharmacological profile of agonists at the benzodiazepine receptor.

Two subtypes of benzodiazepine receptor were suggested originally. The benzodiazepine_I subtype (high affinity for triazolopyridazines) is found throughout the brain with large concentrations in the cerebellum. The benzodiazepine_{II} type was found principally in the cerebral cortex, spinal cord and hippocampus. These distributions were derived from the differential binding of the triazolopyridazine CL 218772, leading to displacement of [³H]benzodiazepine binding, particularly in the hippocampus. Beta carbolines tended to react with benzodiazepine receptors in the cerebellum [54]. In addition, CL 218872 appeared to possess anxiolytic effects with only mild sedation. Therefore, anxiolysis was ascribed to the benzodiazepine_I site [39].

Finally, multiple subunits associated with the receptor labelled with [³H]flunitrazepam were demonstrated [64]. These four proteins had different molecular masses of 51, 53, 55 and 59 kDa. The smaller molecular mass protein had a greater affinity for CL 218872 and this had a different region distribution to the others, a distribution which coincided with the benzodiazepine_I. Cloning experiments have subsequently elucidated further on this heterogeneity of benzodiazepine-GABA_A receptor complexes.

Molecular biology experiments

Experimental studies using specific antibodies raised against subunit variants of GABA_A receptors have shown that the 51 kDa protein, labelled with radiolabelled benzodiazepine, is the $\alpha 1$ subunit: the 53 kDa protein is the $\alpha 2$ subunit and the 59 kDa protein corresponds to the $\alpha 3$ subunit [10, 65]. Pritchett, Luddens and Seeburg [57] analysed the pharmacological characteristics of GABA_A receptors produced by recombining subunits, each moiety containing a single α subunit isoform. They combined different α subunits with $\beta 1$ and $\gamma 2$ in isolated human kidney cells. They then evaluated the binding characteristics of the subsequently formed GABA_A receptors. Receptors containing the $\alpha 1$ subunit behaved like benzodiazepine_I receptors, that is they had high affinity for CL 218872 and the beta carbolines. Those GABA_A receptors which contained $\alpha 2$ or $\alpha 3$ subunits behaved like benzodiazepine_{II} receptors.

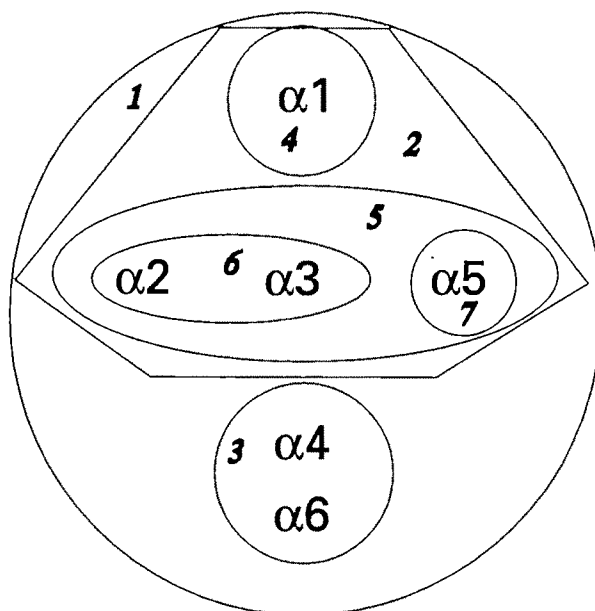


FIG. 3. Venn diagram showing the effect on benzodiazepine pharmacology of the α subunit in combination with $\beta 2$ $\gamma 2$ subunits. Subunits inside the same shape have similar pharmacology (see text for explanation and details).

Further experiments with other α subunits combined with $\beta 2$ and $\gamma 2$ along similar lines revealed a multiplicity of GABA_A receptors and benzodiazepine affinities. These are summarized in figure 3. The largest circle contains α subunits that produce GABA_A receptors which bind muscimol. Polygon 2 contains all those that confer high affinity for diazepam while the circle labelled 3 contains those that have a low affinity for diazepam. The $\alpha 1$ subunit (circle labelled 4) confers benzodiazepine_I pharmacology (i.e. high affinity for CL 218872 and zolpidem). Ellipse 5 contains receptors with benzodiazepine_{II} pharmacology. Those receptors containing the $\alpha 5$ subunit also behaved like benzodiazepine_{II} receptors, but were different from the benzodiazepine_{II} receptors containing $\alpha 2$ and $\alpha 3$ subunits because these recombinant receptors had a different rank order of affinity for zolpidem and CL 218872 [53]. The $\alpha 6$ isoform of the GABA_A receptor has an extremely small binding affinity for benzodiazepines. It is found in the cerebellar granule cell layer [40]. This subtype may be involved in the pharmacological effects of ethanol.

Although this new molecular biological evidence has brought together the pharmacology of GABA and the benzodiazepines, the obvious question to ask concerns the functional significance *in vivo* of these *in vitro* studies. The messenger RNA for the six different α subunits are known and have been mapped within the mammalian central nervous system. It has been shown that the subunits have different topographical distributions [38, 71, 72]. The differences in these distributions may indicate different types of GABA receptor associated with different functions. As yet, this remains to be realized in the development of a drug specially selected for anxiety, lacking sedative and muscle relaxant effects.

Recent pharmacological experiments with the GABA_A antagonist bicuculline indicated separate

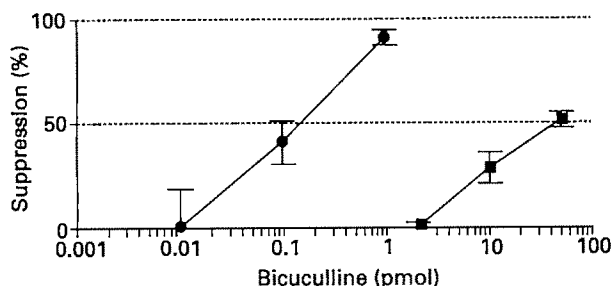


FIG. 4. Bicuculline dose-response curves for suppression of the spinal antinociceptive effects of equipotent doses of intrathecal midazolam (■) and 5-HT (●). Values are mean, SEM.

roles for different GABA_A receptors in spinal antinociception [27]. In these experiments, nociceptive thresholds were measured in the tail of the rat after equipotent intrathecal injections of either 5-HT or the benzodiazepine midazolam. It is known that intrathecal midazolam produces spinally mediated antinociceptive effects by combination with spinal cord benzodiazepine receptors and this may be antagonized by intrathecal bicuculline [22]. Thus midazolam spinally mediated antinociception is mediated by a typical benzodiazepine-GABA interaction. A dose-response curve for bicuculline suppression of midazolam antinociceptive effect revealed that the dose of bicuculline required to produce 50% suppression of midazolam spinal antinociception was approximately 50 pmol (fig. 4) [22].

Similar experiments with 5-HT spinal antinociception revealed that bicuculline could also block the antinociceptive effect of intrathecal 5-HT [27]. However, the dose-response curve for bicuculline in producing this effect was 2.5 decades to the left of that required to block the effects of midazolam (i.e. bicuculline 0.1 pmol was required to produce approximately 50% suppression of 5-HT spinal antinociception (fig. 4)). These widely separated bicuculline dose-response curves may indicate that there exists in the spinal cord two types of GABA_A receptor involved with spinal antinociception. This differential effect of bicuculline on GABA_A receptors has hitherto not been described. Bicuculline tends to be used to define a receptor such as the GABA_A subtype, but no dose-response studies have been performed *in vitro* that confirm or refute this differential sensitivity.

ENDOGENOUS BENZODIAZEPINES

Naturally occurring substances that bind to benzodiazepine receptors were isolated from animals and man as long ago as 1980 [6, 19]. These substances, however, did not become established as endogenous neurotransmitters active at the benzodiazepine receptor because the chemistry used for extraction tended to produce artefacts. The beta carbolines, however, are probably produced in the brain and it is well established that the enzyme systems for their production are present. These substances are inverse agonists that cause anxiety and convulsions when injected into animals. Better extraction systems,

thought not to produce artefacts, have led to the extraction of n-butyl-beta-carboline-3-carboxylate (beta CCB) from bovine brain [45, 51]. This compound varies in concentration in the brain according to stress levels [51]. These data suggest that beta-CCB is an endogenous inverse agonist active at benzodiazepine receptors, perhaps involved in the control of stress.

Agonist substances have also been extracted from brain. These are surprisingly similar to the benzodiazepines developed for use as drugs. For example, De Blas, Park and Friedrich purified desmethyldiazepam from brain extracts [18]. An obvious interpretation of these results is that the animal had somehow ingested benzodiazepines. However, Sangeeswaran and co-workers also detected benzodiazepine immunoactivity in brains of animals and humans that died long before the introduction of the benzodiazepines [61]. These findings have been confirmed by many other groups using different extraction procedures and different tissue sources. The "endozepines" have been purified from rat and human brain [60]. These extracts were shown to modulate GABA activated Cl⁻ conductance in isolated human cortical neurones. The diazepam-like substance has even been reported to be confined to synaptic vesicles [45].

It is still uncertain if these endozepines are naturally occurring neurotransmitters. These substances have been isolated also from common foodstuffs [67, 70] thus throwing further doubt on their neurotransmitter role. However, an *in vivo* mechanism for the formation of 1-4 benzodiazepines has been described [8].

CONCLUSION

Benzodiazepines produce their sedative, hypnotic, anxiolytic, anticonvulsant and antinociceptive effects by interactions with GABA. The benzodiazepines modulate the effects of GABA at the subset of GABA_A receptors. These are ligand-gated chloride ion channels, probably consisting of five subunits. The subunit composition varies in different parts of the brain, although the variations in subunit structure have not been linked with different functions. The type of α subunit determines the benzodiazepine pharmacology—anxiolytic or sedative. Each subunit consists of a chain of amino acids with four hydrophobic segments that span the lipid cell membrane. The intracellular chains joining these membrane-spanning segments may be phosphorylated by intracellular phospholipases. This may lead to modulation of the effect of both benzodiazepines and GABA binding on the cell surface part of the receptor. Many interactions of the benzodiazepine GABA complex with other drugs and neurotransmitters altering intracellular cyclic AMP concentrations are possible. Among these are the opioids, that are well known by anaesthetists to potentiate the sedative effects of the benzodiazepines.

There are many possibilities for different subunit structures of the GABA_A receptors which respond to benzodiazepines. The existence and function of endogenous benzodiazepines is controversial but it

may be that some specialization of GABA_A-benzodiazepine receptors provides more specific targets for these endogenous compounds.

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EXCITATORY AND INHIBITORY SYNAPTIC MECHANISMS IN ANAESTHESIA

G. POCOCK AND C. D. RICHARDS

The administration of inhalation anaesthetic agents is followed by a series of behavioural changes that were first described systematically by Guedel for diethyl ether [49]. The patient passes through stages of analgesia and delirium to reach the unconsciousness of the lighter planes of surgical anaesthesia. The precise pattern of behavioural change varies from agent to agent, and this has caused a great deal of confusion regarding the precise definition of general anaesthesia. In essence, we administer general anaesthetics during surgery to block both the perception of stimuli, particularly painful stimuli, and the accompanying reflex motor responses. General anaesthesia is therefore characterized by loss of consciousness and reduction in reflex activity. As the functional unit of reflex activity is the neurone and as the overall pattern of nervous activity is governed by the functional connections between neurones, we may assume that anaesthetics act by modulating information processing in neural networks. This review is concerned with the mechanisms that underpin this modulation and updates an earlier article on the same subject [109].

In the early part of this century, Sherrington [132] stressed the importance of the synapse in CNS processing. Since then, the details of synaptic transmission within the CNS have gradually been unravelled. The principal events may be summarized as follows: a nerve cell receives information by way of synaptic contacts all over the dendrites and cell body. Impulses travelling in the presynaptic fibres invade the terminal branches to depolarize the nerve endings. This depolarization leads to the opening of the voltage-gated calcium channels and an influx of calcium into the terminal. The subsequent increase in intracellular calcium is the trigger for the exocytotic secretion of transmitter substance from the nerve terminal. The released transmitter diffuses across the synaptic cleft and binds to specific receptor sites on the postsynaptic membrane. Activation of the receptors results in a change in the permeability of the membrane to particular ions, which in turn

leads to a change in membrane potential and to excitation or inhibition of the next neurone of the network, depending on the nature of the synapse under investigation. This brief outline describes the classical view of synaptic transmission as a mediator of fast events, such as spinal reflexes.

Receptors linked directly to ion channels are adapted to fast signalling, but it is now known that synaptic contacts may exert a more subtle long-term modulation of excitability. This modulation operates generally via second messengers such as cAMP and IP₃. For example, noradrenaline interacts with two different classes of receptor, one class activates adenylate cyclase (beta adrenoceptors) and the other inhibits adenylate cyclase (alpha adrenoceptors) [106]. Similarly, the response of muscarinic acetylcholine receptors depends upon the receptor subtype. M₁, M₃ and M₅ receptors activate phospholipase C and generate diacylglycerol and inositol triphosphate (IP₃) while M₂ and M₄ receptor activation is associated with inhibition of adenylate cyclase [40]. Many other CNS transmitters (e.g. dopamine, substance P, 5-HT) are now thought to activate second messenger systems. Nevertheless, both fast and slow synaptic events ultimately modulate specific ion channels and alter the level of excitability of the postsynaptic neurones. The resulting pattern of action potentials encodes information for transmission to the other neurones in the network via other synaptic contacts. Nerve terminals themselves are subject to modulation by presynaptic axonal contacts via presynaptic inhibition [129]. Moreover, axons branch during their course and the spatial pattern of impulses is subject to modulation as changes in threshold at branch points may result in different patterns of impulse activity in different nerve branches. As with alterations to the temporal pattern of activity, changes in the spatial pattern of activity has consequences for information processing by the CNS [113]. The schematic drawing in figure 1 outlines the principal anatomical features that underpin neural processing.

After the early studies of Sowton and Sherrington [139], several workers investigated the actions of anaesthetics on synaptic transmission in the spinal

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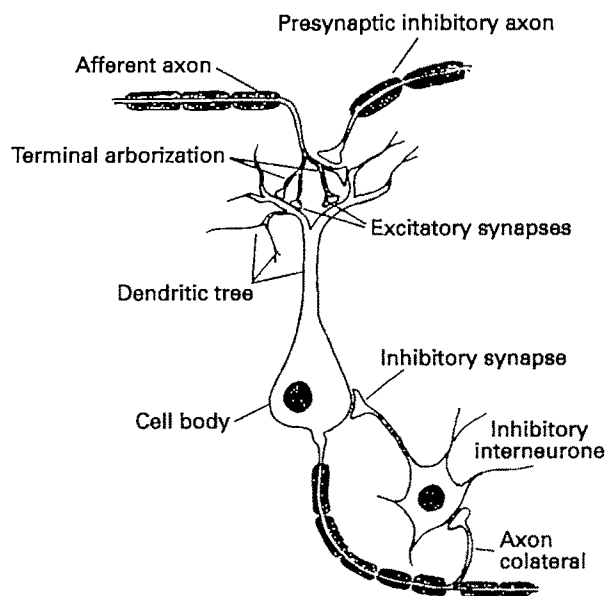


FIG. 1. Schematic diagram of the organization of a synaptic relay within the CNS.

cord and brain. General anaesthetics have been found to depress excitatory synaptic transmission at concentrations likely to be found in the brain during surgical anaesthesia [16, 70, 115, 121, 123, 135, 136, 152]. The effects of anaesthetics on inhibitory synaptic transmission are more varied, as both depression [34, 39, 88, 133] and enhancement [7, 34, 92, 94, 95, 130, 131] have been reported.

From this brief outline of synaptic processing, it is clear that anaesthetics may act at a number of different sites. To reduce transmission through a synaptic relay, anaesthetics may either depress excitation or enhance inhibition. Excitation may be depressed in several ways: by block of action potential propagation, by enhancing presynaptic inhibition, by depressing the release of excitatory transmitters or by depressing the response of the postsynaptic receptors. Anaesthetics may enhance postsynaptic inhibition by increasing the release of inhibitory neurotransmitters or by augmenting the response of postsynaptic receptors. Modulation of synaptic activity results in a change in information processing in the brain. In addition, it is possible that anaesthetics may act directly on postsynaptic neurones by modulating excitability. This could be achieved by increasing directly the threshold for action potential discharge. Alternatively, anaesthetics may modulate the resting conductance of the postsynaptic membrane and so change the membrane potential. Finally, anaesthetics may modulate patterns of action potential discharge, either at the point of initiation or at axon branch points.

To determine precisely how anaesthetics affect information processing in the CNS, we need to investigate systematically the action of anaesthetics on each of the processes mentioned above. We will be concerned chiefly with two questions: (1) What changes in information processing occur during anaesthesia? (2) What cellular and molecular mechanisms are responsible for these changes?

We will focus mainly on the action of anaesthetics

on fast synaptic transmission as this area has received most experimental study.

ACTIONS OF ANAESTHETICS ON EXCITATORY SYNAPTIC TRANSMISSION

Impulse propagation

Do anaesthetics depress excitatory synaptic transmission by block of impulse conduction in the presynaptic nerve fibres? The early studies of Bremer and Bonnet [13, 16] and Larrabee and Posternak [70] showed that impulse conduction in axons was unaffected largely by a variety of anaesthetic agents at concentrations ordinarily required for surgical anaesthesia. Synaptic events, however, were decreased significantly by concentrations of anaesthetics within the clinically relevant range. As Larrabee and Posternak have shown, however, these events were only 5–10 times more sensitive to inhibition than impulse conduction. Thus in comparison with very specific blockers of synaptic transmission, such as atropine, curare or propranolol, anaesthetics are not very selective inhibitors of synaptic activity. More recent work by Somjen [135, 136], Weakly [152] and Richards and co-workers [115, 117, 118, 121, 123, 124] has confirmed that synaptic transmission in the CNS is significantly more sensitive to depression by most anaesthetic agents than is action potential propagation.

Although this evidence suggests strongly that a decrease in action potential propagation is unlikely to occur in nerve trunks during general anaesthesia, some workers have suggested that a reduction in action potential amplitude in nerve terminals may lead to a decrease in excitatory synaptic transmission [9, 36]. If this were so, in carefully chosen experimental situations it should be possible to mimic the effects of general anaesthetics by the application of nerve blocking agents, such as procaine or tetrodotoxin. Detailed examination of the synaptic potentials evoked by electrical stimulation of afferent pathways in the olfactory cortex and hippocampus has shown that a wide variety of general anaesthetics depress excitatory synaptic transmission with no significant change, either in the amplitude of the focally recorded action potential or in the latency of onset of the excitatory postsynaptic potentials (EPSPs) [9, 115, 117, 121, 124]. Similar experiments performed with nerve blocking agents show changes in both, as expected [118] (see fig. 2).

At large concentrations of anaesthetic, such as those experienced briefly during induction of general anaesthesia, propagation of impulses may be impaired, especially at points with small safety factors, such as fine axon branches. Evidence that this may occur has recently come from work by Berg-Johnsen and Langmoen [9] who found that impulse conduction in the small unmyelinated fibres (0.1–0.2 μm diameter) of the rat hippocampus was far more sensitive to block by isoflurane than impulse conduction in the myelinated fibres of the fimbria (1 μm diameter). If such a block were to occur at small concentrations of other anaesthetics, it would contribute to depression of excitatory synaptic transmission. Although this does not appear to be the case

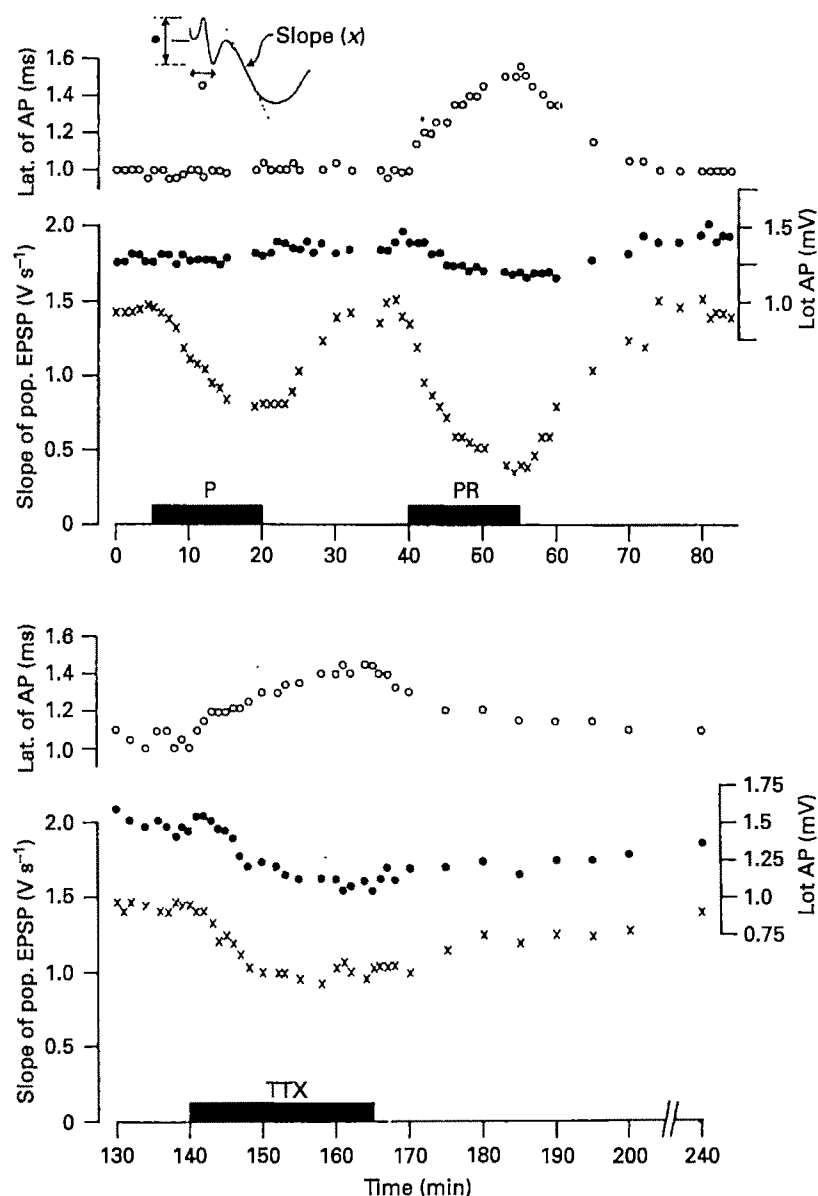


FIG. 2. Comparison of the effects of pentobarbitone (P) $0.3 \text{ mmol litre}^{-1}$, procaine (PR) $0.5 \text{ mmol litre}^{-1}$ and tetrodotoxin (TTX) $2 \times 10^{-8} \text{ mol litre}^{-1}$ on impulse conduction and synaptic transmission in the olfactory cortex. The inset figure shows how the amplitude and latency (Lat.) of the lateral olfactory tract action potential (Lot AP) were measured (filled and open symbols, respectively). The rate of rise of the EPSP field potential (pop. EPSP) is used as an index of excitatory synaptic transmission (X) (reproduced from [118] with permission).

for the majority of clinically useful agents, there is evidence that this mechanism may contribute to the actions of agents in addition to isoflurane [77, 125]. For example, large concentrations of propanidid ($0.3 \text{ mmol litre}^{-1}$) depress synaptic transmission in the olfactory cortex, and parallel with this effect is a small decrease in action potential amplitude and an increase in its latency [125]. The actions of anaesthetics on human sodium channels incorporated into artificial bilayers have been studied by Urban and colleagues [146]. Most general anaesthetics produce little modulation of sodium channel activity at concentrations likely to be found in the brain during anaesthesia, but propofol is an exception and may have a similar action to propanidid and isoflurane.

While there is no doubt that general anaesthetics reduce action potential propagation in nerve axons, the point at issue is whether or not this effect is responsible principally for the reduction in ex-

citatory synaptic transmission. From the evidence discussed above, it appears that, during general anaesthesia, most anaesthetics act mainly to disrupt the process of chemical transmission and do not depress excitatory synaptic activity by a partial block of impulse conduction in the presynaptic nerve axons.

If anaesthetics do not depress excitatory synaptic transmission via block of impulse conduction, how do they act? Clearly, to depress excitatory synaptic transmission, anaesthetics must decrease the release of transmitter from presynaptic nerve terminals, depress the sensitivity of the postsynaptic receptors to released transmitter, or both.

Anaesthetic action on the release of neurotransmitters

The early work of Matthews and Quilliam [84] showed that clinically relevant concentrations of amylobarbitone decreased the amount of acetyl-

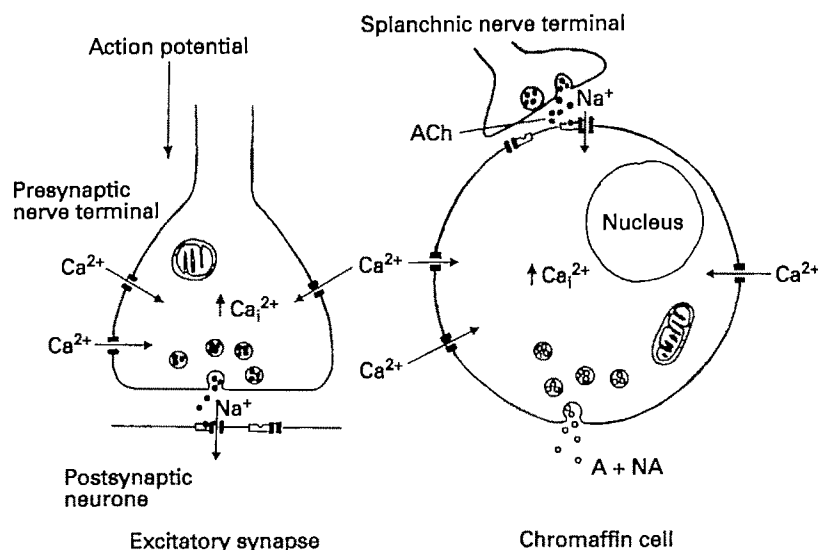


FIG. 3. Schematic diagram indicating the parallel between the secretion of catecholamine from a chromaffin cell and the activation of an excitatory synapse. Calcium ions are represented as moving through voltage-gated channels. A = Adrenaline; NA = noradrenaline; ACh = acetylcholine. Adapted from [110].

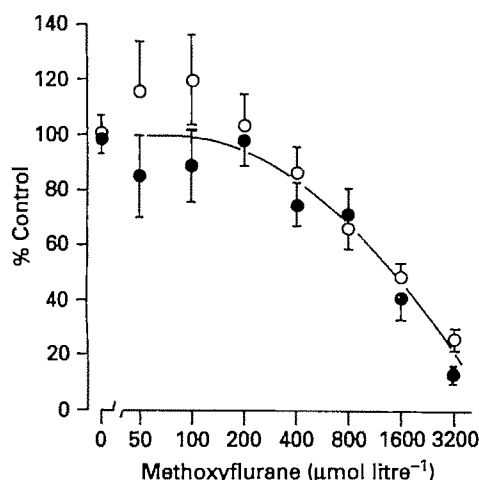


FIG. 4. Dose-response relationship for the effect of methoxyflurane on K^{+} -evoked catecholamine secretion (●) and calcium uptake (○) of isolated chromaffin cells. Note the close parallel between the inhibition of evoked calcium uptake and the inhibition of catecholamine secretion. Value are means, SEM.

choline released from sympathetic ganglia by electrical stimulation of the preganglionic nerves. Other central depressants had a similar effect. The excitatory amino acids aspartate and glutamate, are thought to be the main transmitters for fast excitatory synaptic transmission in the CNS. Anaesthetics have been shown to depress the release of glutamate and aspartate evoked from brain tissue slices by direct depolarization with potassium [25, 64, 87, 111]. The extent of the decrease in transmitter secretion is dose-dependent, although not all agents exert a powerful depressant action. Unfortunately, these studies cannot simply be taken at face value as depolarization by potassium also reverses electrogenic transport of glutamate [62]. More unambiguous data concerning the action of anaesthetics on neurosecretion have been obtained from studies on adrenal chromaffin cells. These cells are homologous with sympathetic postganglionic neurones and the potassium-evoked secretion of

catecholamines is analogous to neurosecretion caused by invasion of the nerve terminal by an action potential (see fig. 3). A wide variety of anaesthetic agents have been found to depress the catecholamine secretion evoked by potassium, both in intact adrenal glands [45, 46, 57] and in isolated chromaffin cells [107, 108] (fig. 4). As discussed below, chromaffin cells have provided a valuable experimental model in which to investigate the action of anaesthetics on neurosecretion in great detail.

Electrophysiological evidence that barbiturates cause a reduction in the release of neurotransmitters from central synapses comes from a study by Weakly [152] who found that small doses of thiopentone and pentobarbitone decreased the quantal content of EPSP in motor neurones without changing the amplitude of miniature EPSP. Ether [160], and halothane and thiopentone [69] have subsequently been reported to have a similar action. This work will be discussed further when the relative importance of presynaptic and postsynaptic effects of anaesthetics is assessed.

How do anaesthetics inhibit the release of neurotransmitter substances from nerve terminals? It is unlikely that decreases in transmitter release can be attributed to alterations in the content of neurotransmitters within the nerve terminals, as the total content either remains unchanged (for example, glutamate, aspartate and GABA [111]) or is increased, as in the case of acetylcholine [28]. Nevertheless, Bangham and Mason [5] have suggested that anaesthetics may deplete vesicular transmitter stores by dissipating the proton gradient across their surface membrane. In answer to this possibility, Akeson and Deamer [2] have shown that anaesthetics do not cause major depletion of catecholamines from isolated chromaffin granules. Furthermore, this mechanism would predict increased leakage of catecholamines from chromaffin cells rendered leaky to small molecules (e.g. by exposure to high voltage electrical discharge) (electroporabilized cells). The experimental evidence, however, does not support this

view, as Pocock and Richards [107, 108, 110] have found that a variety of anaesthetics did not increase significantly the leakage of catecholamines from either intact or electroporabilized cells. The weight of evidence, therefore, suggests that anaesthetics do not deplete presynaptic vesicular stores of transmitter.

After an action potential depolarizes the nerve terminal, there is an increase in free calcium which is believed to trigger the release of transmitter [4, 63, 143]. As discussed above, calcium enters the nerve terminal through voltage-gated calcium channels (see fig. 3). Anaesthetics could either depress the evoked transmitter secretion by a direct effect on these channels or disrupt the intracellular events leading to exocytosis. Using electroporabilized chromaffin cells, Pocock and Richards [107, 108, 110] found no evidence to suggest that anaesthetics altered the relationship between intracellular free calcium and catecholamine secretion. Moreover, since the depression of potassium-evoked catecholamine secretion caused by most anaesthetic agents was paralleled closely by inhibition of calcium influx (e.g. fig. 4), they concluded that anaesthetics inhibit the secretion of catecholamines by inhibiting calcium entry. Data consistent with this interpretation have recently been reported for the action of volatile anaesthetics on stimulus-secretion coupling in PC12 and GH3 tumour cell lines [67, 140]. In addition, barbiturates depress the potassium-evoked calcium influx of synaptosomes [12].

In chromaffin cells, halothane [108, 156] had little depressant effect on either potassium-evoked calcium influx or catecholamine secretion of chromaffin cells, except at large concentrations ($> 1 \text{ mmol litre}^{-1}$). A similar finding has recently been reported for the action of halothane on the potassium-evoked efflux of aspartate from cerebral cortex slices [3]. These findings raise some difficulties in accounting for the depressant action of halothane on synaptic transmission. At many synapses, including those of the olfactory cortex and hippocampus, glutamate is the putative transmitter and yet halothane has no significant depressant effect on the sensitivity to glutamate [26, 122]. Ketamine [142] also has little inhibitory effect on potassium-evoked catecholamine secretion from chromaffin cells or on potassium-evoked transmitter release from synaptosomes [103].

In contrast to the action of halothane on chromaffin cells and brain slices noted above, Kress and co-workers [67] found that halothane inhibits both catecholamine secretion and the increase in intracellular free calcium that follows depolarization of PC12 cells, with IC_{50} values of approximately $0.75 \text{ mmol litre}^{-1}$. Differences in the properties of the calcium channels of the various tissues may account for the difference in sensitivity to halothane. Kress and co-workers [67] noted that the voltage-gated calcium channels of transformed cells may not be modulated in the same way as those of nerve endings or primary neurosecretory cells, such as chromaffin cells. They point out that in heart muscle, the L type calcium channels are modulated by a cAMP and Ca-dependent protein kinase [30], while

the L type calcium channels of PC12 cells and chick dorsal root ganglion cells are modulated by protein kinase C, which is activated by diacylglycerol [99].

From the evidence reviewed above, we conclude that most general anaesthetics depress the depolarization-induced secretion of transmitters. This decrease in transmitter secretion is caused by inhibition of calcium influx through voltage-gated calcium channels. Anaesthetic modulation of the exocytotic machinery does not appear to be important.

Anaesthetic action on calcium currents. The release of neurotransmitters and many other aspects of neural function are regulated by calcium ions [4, 65, 81, 143]. The action of anaesthetics on calcium currents is therefore of some importance in understanding the mechanism of anaesthesia. The first studies were made in *Helix* neurones [97, 98] where pentobarbitone was found to inhibit voltage activated calcium currents by increasing the rate of voltage-dependent inactivation. Later, pentobarbitone was found to decrease calcium currents in cultured dorsal root ganglion cells [154]. These observations are consistent with the depressant action of anaesthetics on calcium influx, but were made before the various subtypes of calcium channels had been described. We now know that there are at least four types of voltage-gated calcium channels, L, N, P and T. The L, N and T subtypes were described first in chick dorsal root ganglion cells [101]. The P channels were described later and are found in cerebellar Purkinje cells [71]. The different subtypes may be distinguished by sensitivity to various inhibitors and by electrophysiological characteristics. The role of the different subtypes in regulating neural function is still being clarified, but N type channels have been implicated in the regulation of transmitter release [86]. Gross and MacDonald [48] examined the action of pentobarbitone on the calcium currents of cultured dorsal root ganglion neurones and found that while pentobarbitone affected the large threshold calcium currents it had little effect on the small threshold T current. The rate of inactivation of the large threshold currents was increased and the amplitude depressed. Herrington and colleagues [55] investigated the action of halothane on the calcium currents of GH3 cells and found that, as with the action of pentobarbitone on dorsal root ganglion neurones, halothane had little effect on T currents but inhibited the large threshold L currents with an IC_{50} value of $0.5 \text{ mmol litre}^{-1}$. In adrenal chromaffin cells, currents caused by both N and L channels have been observed [14, 15, 22, 53] but no currents caused by T channels were found. Charlesworth, Pocock and Richards [23] have shown that methohexitone and methoxyflurane inhibit directly the currents caused by activation of calcium channels of the N and L types at concentrations similar to those required to inhibit the calcium influx evoked by direct depolarization. There was no evidence to suggest a selective action on either N or L channels. The local anaesthetic procaine, was much less effective in depressing voltage activated calcium currents in accord with its weak inhibitory effect on potassium-evoked catecholamine secretion [21].

Effects of anaesthetics on excitatory postsynaptic receptors

As we have mentioned previously, in addition to depressing transmitter secretion, anaesthetics may decrease excitatory synaptic transmission by actions on postsynaptic receptors. Early studies showed that anaesthetics depress the response to applied excitatory agonists [7, 26, 27, 122]. In this section, we consider the actions of anaesthetics on fast excitatory mechanisms and on slow synaptic modulation, exemplified by muscarinic excitation of cortical neurones.

The amino acids aspartate and glutamate, are thought to be important excitatory neurotransmitters in the CNS [59, 68, 150] and the action of anaesthetics on their receptors has received attention. Early studies by Crawford and Curtis [27] showed that barbiturates reduced the sensitivity of neurones in the cerebral cortex to iontophoretically applied glutamate. Later studies confirmed this finding and showed that this was also true for other anaesthetic agents [26, 121, 122, 128]. These receptors, however, were resistant to depression by halothane at concentrations within the anaesthetic range [26, 122].

Since these studies were performed, at least three subtypes of ionotropic glutamate receptor have been described and named after their preferred agonists: N-methyl-D-aspartate (NMDA), kainate and quisqualate [150, 151]. More recently, the quisqualate receptor has been renamed the AMPA receptor as AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) has been found to be a much more specific agonist [151]. At glutaminergic synapses, the AMPA and kainate subtypes are thought generally to mediate fast excitatory synaptic transmission and NMDA to mediate slower events, including modulation of synaptic efficacy [85]. In addition to the ionotropic receptors, a metabotropic glutamate receptor linked to a G protein has been described which, when activated, leads to an increase in intracellular IP₃ and diacylglycerol [85].

To date, there has been little detailed investigation of the action of anaesthetics on the subtypes of excitatory amino acid receptor. Ketamine, phencyclidine, ethanol and diethyl ether have been shown preferentially to depress responses of the NMDA subclass [72, 73, 153], whereas barbiturates depress the sensitivity of neurones to all three agonists with quisqualate/kainate activation showing the greatest sensitivity [128, 153]. Oocytes injected with mRNA from rat brain express kainate receptors and the response of these cells to kainate is depressed by phenobarbitone [31].

In an attempt to discover the general rules by which anaesthetics influence receptor function, Quastel and Gage and their colleagues have made a detailed study of the decay of end-plate currents [41, 42, 104]. Both groups found that many anaesthetics prolonged the time course of the decay phase of the current. Detailed study of the effects of a variety of agents revealed significant differences in the manner by which different anaesthetics modulate the decay of end-plate currents [104] and suggests

that not all agents act in precisely the same way. Various models of receptor block by anaesthetics have been proposed and will be discussed later.

Neuronal nicotinic acetylcholine receptors are now known to occur throughout the CNS and many isoforms have been identified using the techniques of molecular biology [127]. The nicotinic receptor found in bovine chromaffin cells is similar in its physiological properties to the nicotinic receptor of sympathetic ganglion cells [29, 82, 83] and anaesthetics exert a powerful depressant effect on the catecholamine secretion of chromaffin cells that is evoked by nicotine agonists [45, 46, 57, 107, 108, 142]. The inhibition of carbachol-evoked catecholamine secretion by pentobarbitone and methoxyflurane is non-competitive and is associated with inhibition of the evoked influx of both sodium and calcium ions [107, 108].

Acetylcholine receptors in the brain are predominantly muscarinic [68] and are thought to mediate slow synaptic excitation and inhibition by modulating potassium conductances [18, 47, 50]. The effects of anaesthetics on this class of acetylcholine receptor have been studied by Smaje [134] who showed that volatile anaesthetics enhanced the response to ionophoretically applied acetylcholine whereas alphaxalone depressed it. Pentobarbitone had no consistent effect on muscarinic excitation. More recently, Puil and el Beheiry [112] found that pentobarbitone and isoflurane inhibited the muscarinic response of neocortical neurones. The reason for this apparent discrepancy is not clear, but we now know that at least five types of muscarinic receptor exist in the brain (M1–M5). Activation of the odd series (M1, M3, M5) activates phospholipase C and leads to the generation of IP₃. Activation of the even series (M2, M4) leads to inhibition of adenylate cyclase [40]. In some neurones, M2 muscarinic receptor activation inhibits M type potassium channels and causes depolarization [18, 106]. There is at present no information concerning the interaction of anaesthetics with specific subtypes of the muscarinic receptor and this is an important area for future study.

ANAESTHETIC ACTIONS ON INHIBITORY SYNAPTIC TRANSMISSION

The actions of anaesthetics on inhibitory synaptic transmission are more complex than those on excitatory synaptic transmission. In general, barbiturates have been found to enhance both pre-synaptic and postsynaptic inhibition [7, 34, 44, 90, 92, 94, 95], although other anaesthetics, such as ether, may depress inhibition [34, 39, 158]. Recent studies on brain stem neurones have described a variety of responses to the action of general anaesthetics on synaptic mechanisms [133]. Halothane depresses predominantly both excitation and inhibition [133, 158], while barbiturates appear to depress excitation but enhance inhibition [133]. The variable effects of anaesthetics on synaptic inhibition probably reflects the fact that excitation of inhibitory interneurones within nerve networks is potentially susceptible to anaesthetic block. This block would

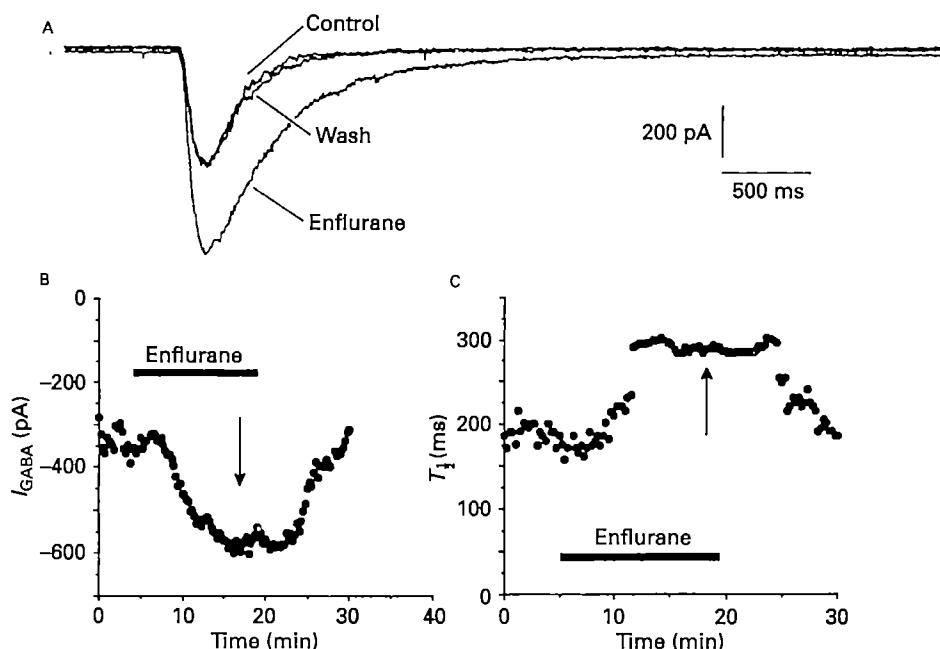


FIG. 5. Effect of enflurane $0.58 \text{ mmol litre}^{-1}$ on GABA activated chloride currents in cultured hippocampal neurones (A). Enflurane increases the amplitude of the chloride current from approximately -350 pA to approximately -600 pA (B) and increases the time constant for current decay (C) (reproduced with permission from [61]).

prevent the synaptic activation of inhibitory interneurons.

In spite of the apparent complexity of the effects of anaesthetic on inhibition in the CNS, the cellular mechanisms that underpin both excitation and inhibition are essentially the same. The difference between excitatory and inhibitory synapses resides in the different nature of their transmitters and in the characteristics of the inhibitory receptor-channel complex. The fact that anaesthetics may enhance or depress inhibition depending on the pathway under consideration is inconsistent with the idea that general anaesthetics depress synaptic transmission by generalized block of action potential propagation in nerve terminals.

Anaesthetic action on the release of inhibitory transmitters

In the CNS the major inhibitory neurotransmitters are thought to be the amino acids GABA and glycine: GABA is the predominant inhibitory transmitter in the brain and glycine predominates in the spinal cord [68]. While little is known of the effects of anaesthetics on synapses utilizing glycine as a neurotransmitter, the action of anaesthetics on synapses thought to utilize GABA has received considerable attention.

As the underlying mechanism of neurosecretion is believed to be the same whatever transmitter is being secreted, it is not surprising that general anaesthetics have a depressant action on the release of GABA, the principal inhibitory transmitter in the brain [60, 64, 87]. This is consistent with the action of anaesthetics on the secretion of excitatory transmitters. Nevertheless, some authors have reported that anaesthetic concentrations of barbiturates increase GABA secretion [25, 111]. The reasons for this discrepancy are not clear but, in intact brain tissue, the interpretation of such studies is com-

plicated by the fact that these amino acids are involved widely in cerebral metabolism.

Action of anaesthetics on inhibitory postsynaptic receptors

It is probable that anaesthetics depress neurotransmitter release at both excitatory and inhibitory synapses, but this is yet to be clarified fully (see above). If this is true, however, the enhancement of inhibition may be attributed to interactions between anaesthetic agents and the postsynaptic GABA receptor-channel complex. GABA receptors are now known to be of two classes: GABA_A receptors possess an integral chloride channel and mediate the fast inhibitory postsynaptic potentials (IPSP) while GABA_B receptors mediate slower late IPSP and appear to be G protein linked metabotropic receptors. These interactions of anaesthetics with what are now classified as GABA_A receptors have received considerable attention in recent years. Pentobarbitone has been shown to prolong and intensify the hyperpolarizing action of GABA in frog motoneurons [93] and in mouse cortical neurones [7], and alphaxalone has been reported to have a similar action [6]. This increase in the effect of GABA does not result from a decrease in uptake [60] and is probably the result of increase in chloride conductance. In agreement with this, Jones, Brooks and Harrison [61] have shown that enflurane, halothane and isoflurane augment the chloride currents evoked by application of GABA (see fig. 5). In addition, enflurane has been found to modulate the response of hippocampal neurones to GABA in a biphasic manner: slow responses to exogenously applied GABA were blocked while rapid responses were potentiated [17, 90]. Measurements of chloride channels activated by GABA in single chromaffin cells have supported the view that anaesthetics increase chloride conductance [76, 105] and have

shown that barbiturates could activate directly chloride conductances in some cells, a finding confirmed recently by others [58].

HOW DO ANAESTHETICS MODULATE RECEPTOR FUNCTION?

There are three possible mechanisms by which anaesthetics may depress the response to excitatory agonists: they may desensitize the receptor in some manner and reduce the number of active channels, they may reduce the specific conductance of the channels or they may reduce the time for which the channels are open. Any combination of these three mechanisms is also possible. There is evidence from studies on the nicotinic receptor of *Torpedo electrophorus* [35, 54, 159] that certain anaesthetics may desensitize the receptor to acetylcholine. For barbiturates, this appears to result from an increase in high affinity binding of acetylcholine to the receptor from which it is slow to dissociate.

In contrast to their depressant effects on excitatory synaptic transmission, barbiturates generally augment the response to GABA and increase the affinity of the GABA receptor for GABA by an allosteric effect which modulates the kinetics of the associated ion channel [102, 144]. This view has received some support from recent biophysical studies of GABA channel kinetics (see below). The effects of anaesthetics on the affinity of other neurotransmitter receptors are not known.

Anaesthetic modulation of ion channel kinetics

Adams [1] showed some years ago that barbiturates and procaine depressed the second of a pair of closely spaced end-plate potentials much more effectively than the first. He proposed that this occurred because the anaesthetics blocked preferentially ion channels in the open conformation. This model proposes that anaesthetics enter the open channel and block it. The blocked channel must then open before it can return to the closed state. This is called the sequential blocking model and a number of predictions about the kinetics of channel block may be derived from this model [21, 43]. These predictions can be tested readily using patch-clamp recording techniques [52]. These predictions are met largely when the acetylcholine receptor of the neuromuscular junction is exposed to small concentrations of the lignocaine derivative QX 222 [91]. The model does not, however, account for the effect of barbiturates [43] on these channels or for the action of ketamine, volatile anaesthetics and alcohols on the nicotine receptors of mouse tumour cell lines [32, 147–149]. While most anaesthetics did not reduce the specific conductance of the channel, short chain alkanols did (fig. 6). Dilger and Brett [32] have proposed a modification to the sequential blocking model which permits the blocked channel to enter a further long-lived blocked state (see also [89]). This extended block model accounts for much of the action of anaesthetics on the muscle nicotinic receptor, including the apparent reduction in single channel amplitude which results from a rapid alteration between the open and blocked states that is

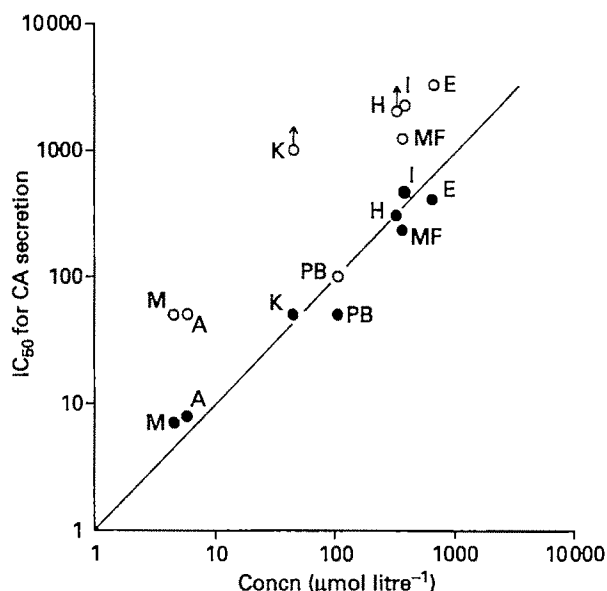


FIG. 6. Correlation between the concentration of anaesthetic required for general anaesthesia and the IC_{50} ($\mu\text{mol litre}^{-1}$) for inhibition of catecholamine (CA) secretion evoked by potassium (○) and carbachol (●). Note that the IC_{50} values for carbachol-evoked secretion cluster more closely around the line of identity than the IC_{50} values for direct depolarization. M = Minaxalone; A = alphaxalone; K = ketamine; PB = pentobarbitone; H = halothane; MF = methoxyflurane; I = isoflurane; E = enflurane. (Data from [107, 108, 142] and unpublished experiments.)

too fast to resolve with present recording techniques [32].

It may be argued that, as general anaesthetics do not produce muscle paralysis, their interaction with the nicotinic channels of muscle may not be representative of their effects on other agonist-operated channels, as it is well known that neurones possess a different type of nicotinic receptor to that of muscle. Neuronal nicotinic receptors are found on adrenal chromaffin cells and are readily accessible to experimental investigation. Patch-clamp studies of this receptor show a pattern similar to that for the neuromuscular receptor. Pentobarbitone [58] and procaine [21] reduced the time for which the channels were open. Detailed analysis of the action of procaine suggested that neither the sequential blocking model nor the extended block model would adequately explain how the anaesthetic reduced the lifetime of the open channel [21].

As we have already indicated, fast synaptic excitation in the CNS is attributable, to a large extent, to glutaminergic synapses. The action of anaesthetics on the subtypes of the glutamate receptor is beginning to be explored. Small concentrations of isoflurane have been reported to decrease the probability of channel opening without a significant effect on the mean open time of channels gated by NMDA, but greater concentrations ($> 1 \text{ mmol litre}^{-1}$) also reduce mean open time [155]. Alkanols have similar effects [80]. Dissociative anaesthetics, such as ketamine, appear to block open NMDA channels preferentially and this block is both voltage- and use-dependent [51, 56, 74, 75]. In this case, it is proposed that the channel closes while the anaesthetic is bound. Dissociation of the anaesthetic from the blocked state is very slow and the

number of blocked channels accumulates as the preparation is activated repeatedly [51, 75]. Use-dependent local anaesthetic block of open voltage-gated sodium channels operates in a similar way [20, 141]. Detailed kinetic analyses of the action of other anaesthetics on NMDA receptors are needed as are studies of the action of anaesthetics on other glutamate receptor subtypes.

One of the most dramatic and remarkable effects of many anaesthetics is their potentiation of GABA-mediated inhibition. It is now clear that this potentiation results from a direct action of anaesthetics on GABA activated channels. Two studies have investigated the anaesthetic modulation of GABA_A channel kinetics. MacDonald, Rogers and Twyman [76] found evidence to suggest that GABA_A channels have three different modes of opening and that pentobarbitone appeared to increase the relative frequency of the opening mode with the longest duration. This has the effect of increasing the mean open time of the channel and prolongs the duration of groups of openings known as "bursts" [76]. Halothane has been reported to show a similar action [157].

ANAESTHETICS AND SECOND MESSENGER SYSTEMS

Clearly, anaesthetics as a group, affect profoundly the function of ion channels at concentrations within the clinical range. The depressant effects of anaesthetics may be explained by a decrease in the time for which excitatory channels remain open, while the enhancement of inhibition may be explained by an increase in channel conducting time. These two effects are mutually reinforcing.

Although the detailed mechanisms remain to be resolved, it is becoming increasingly clear that the effects of anaesthetics on synaptic transmission may be explained best by direct interactions between the anaesthetics and the channel proteins [32, 89, 149], in line with earlier hypotheses of anaesthetic action [37, 119, 120]. This suggests that ion channels may represent a major target for anaesthetic molecules. Nevertheless, many channels, including calcium and potassium channels, are modulated by phosphorylation by protein kinases [114] and by systems linked to second messengers, such as cAMP and cGMP [33]. For these channels, it is important to determine if anaesthetics act directly or via modulation of second messenger systems. For example, MacIver, Tanelian and Mody [79] have suggested that pentobarbitone and halothane may in fact act via separate mechanisms to enhance GABA_A activated chloride conductances in hippocampal neurones. Halothane may act to prolong spontaneous inhibitory postsynaptic currents as a consequence of an increase in intracellular calcium, as its actions are blocked if cells are filled with the calcium chelator BAPTA. In contrast, the effects of pentobarbitone could not be blocked by BAPTA and were attributed to direct interactions between the anaesthetic and the channel.

GH3 cells are of interest as TRH-evoked prolactin secretion has two components: an early component as a result of activation of phospholipase C and a later component as a result of opening of voltage-

gated calcium channels. Activation of phospholipase C leads to the generation of two second messengers, diacylglycerol and IP₃. The IP₃ causes release of calcium from stores within the cell and triggers secretion. Stern and colleagues [140] have examined the action of halothane on GH3 cells and found that the early IP₃ activated increase in intracellular free calcium (Ca_i) was not inhibited readily by halothane, while the component of the Ca_i increase as a result of calcium influx was inhibited with an IC₅₀ value of 0.4 mmol litre⁻¹. The insensitivity of the IP₃ activated increase in intracellular free calcium to halothane reported by Stern and co-workers [140] is supported by the work of Kress and colleagues [67] on PC12 cells. In these cells, as elsewhere [24, 40], muscarinic stimulation induces secretion by increasing IP₃ which in turn increases intracellular calcium. Kress and co-workers found that muscarinic stimulation of PC12 cells was not inhibited by a variety of volatile anaesthetics.

Some time ago it became clear that while anaesthetics did not reduce cerebral energy concentrations measured by ATP and creatine phosphate [11], they did modulate the concentrations of cyclic nucleotides [145] at least at large concentrations. The significance of these changes in modulating neural function is yet to be established, but such interactions clearly require further investigation.

ANAESTHETIC ACTIONS ON POSTSYNAPTIC EXCITABILITY

In principle, in addition to their synaptic actions discussed above, anaesthetics may modulate the electrical excitability of neurones directly. Early studies indicated that anaesthetics had little effect on the threshold for action potential generation [115, 117, 135, 136, 152] or on the resting membrane potential [123, 128, 136, 152]. There is some evidence, however, that certain general anaesthetics may modulate the ionic conductances of the resting membrane of CNS neurones [10, 38, 93, 96], this effect being particularly notable with the barbiturates [93, 96] and isoflurane [10, 138]. In addition to these effects on the resting membrane, some anaesthetics appear to block accommodation in hippocampal neurones. This property is shown by enflurane, halothane, isoflurane and ketamine [78, 137, 138] and is probably mediated, at least in part, by modulation of potassium channel activity [38, 50]. This action may account for the reported increase in excitability of some neurones in the presence of isoflurane and enflurane [8, 78] and for the changes in firing pattern described by Fujiwara and co-workers [39] and earlier authors [100, 116, 121, 126].

CHANGES IN THE PATTERN OF AXONAL ACTIVITY

The widely accepted view that anaesthetics disrupt synaptic transmission does, however, ignore the possibility that they may exert more subtle influences on the temporal and spatial pattern of impulse propagation. As Raymond, Shin and Stefensen pointed out [113], the passage of a nerve impulse produces oscillations in membrane excitability and

alterations in the conduction velocity of succeeding impulses, which may last for an appreciable time. This being so, impulse conduction through axon branch points may affect nerve coding as the subsets of terminals invaded by a nerve impulse may vary, depending on the local level of excitability. This possibility has been investigated by Raymond and colleagues [19, 113] who found that while small concentrations of halothane and enflurane increase the threshold for impulse conduction they *depress* the increase in threshold that follows the passage of short high frequency trains of nerve impulses. Moreover, certain anaesthetics inhibit sodium currents more effectively during repetitive stimulation and so produce a use-dependent block of impulse conduction that is more effective during high frequency trains of impulses [141]. As the unit of information transfer between neurones is the action potential and as the information processing unit is the neurone with its extensive synaptic inputs, modulation of the pattern of impulses in the presynaptic fibres by anaesthetics will have major importance for information processing in neural networks and would be additional to the anaesthetic-induced changes in the pattern of impulses during synaptic activation, (see ANAESTHETIC ACTIONS ON POSTSYNAPTIC EXCITABILITY above).

ARE THE EFFECTS OF ANAESTHETICS PRINCIPALLY PRE- OR POSTSYNAPTIC?

In assessing the action of anaesthetics on synaptic transmission, it is important to establish the relative importance of presynaptic and postsynaptic effects. At a chemical synapse it is generally accepted that there are a number of sites n at which the release of a relatively fixed amount of transmitter q occurs with a probability P . The response of the postsynaptic membrane is therefore related to the product nPq . The evidence from quantal analysis of spinal motoneurone excitatory postsynaptic potentials (EPSP) would appear to suggest that anaesthetics act predominantly at presynaptic nerve endings [69, 152, 160]. This implies that when anaesthetics depress EPSP, they reduce n or P and a change in either or both of these variables may be reconciled easily with inhibition of calcium influx. The quantal analysis of changes in synaptic efficacy due to application of anaesthetics is, however, based on fluctuation analysis of EPSP amplitude and assumes that the fluctuations are discrete. For technical reasons this assumption remains questionable, especially where the signal-to-noise ratio is poor (see Korn and Faber [66]). Although the conclusions drawn from quantal analysis cannot be accepted as clear proof of a dominant presynaptic site of action for anaesthetics, this form of analyses remains the only way of determining the relative importance of pre- and postsynaptic modulation. Further work in this area is needed.

In addition to the technical difficulties alluded to above, the specific features of particular synapses (e.g. the precise type of calcium channel present in the presynaptic membrane or the number and nature of the postsynaptic receptors) may also determine if

the effects of an anaesthetic are predominantly pre- or postsynaptic. Consider first a synapse where the amount of transmitter released fully saturates the available receptors. Any anaesthetic-induced modulation of receptor function (e.g. a reduction in mean channel lifetime) will have an immediate consequence. For a reduction in transmitter release to have a similar impact, the excess release must first be eliminated. Conversely, in the situation where there are many spare receptors, a reduction in neurosecretion will have a profound effect on synaptic efficacy. Nevertheless, even in this situation, a change in receptor function will contribute to the overall effect unless transmitter release fails completely.

By considering the relative susceptibility of pre-synaptic and postsynaptic processes to modulation by anaesthetics, we may infer the relative importance of these processes as targets of anaesthetic action. Using the chromaffin cell model, it appears that neurosecretion evoked by depolarization with potassium is less susceptible to depression by general anaesthetics than the events linked to activation of the nicotinic receptor (see fig. 6) [107, 108, 156]. This would suggest that anaesthetics act principally at the postsynaptic level. Comparable neurochemical evidence from nerve terminals is lacking. At present, therefore, there is insufficient information to provide a clear answer to this problem.

SUMMARY

To understand the cellular and molecular basis of the anaesthetic state, it is important to remember that, in the intact CNS, synapses operate within elaborate nerve networks. From the data presented above, it is evident that block of impulse conduction in presynaptic fibres does not explain the effects of most anaesthetics on synaptic activity. This is not surprising since some anaesthetics, the barbiturates in particular, may both depress excitation and enhance inhibition. General anaesthetics modulate the activity of presynaptic voltage-gated calcium channels and this appears to be sufficient to account for the reduction in transmitter secretion they produce. Transmitter operated ion channels in the postsynaptic membrane are modulated by smaller concentrations of anaesthetics than are required to modulate the presynaptic voltage-gated calcium channels. For this reason, transmitter operated channels appear to represent a major target site for anaesthetics.

Finally, there are subtle effects of anaesthetics on the patterns of impulse propagation in nerve axons and on action potential generation in the cell body which result from modulation of membrane excitability. The overall effect of an anaesthetic agent depends on summation of events occurring at the many individual synapses and neurones that make up the network. The effects of anaesthetics on different neuronal pathways may therefore depend on the nature of the receptors and ion channels of the cells that comprise the network. The anaesthetic state may be the result of all these actions, but the characteristics of the state may differ somewhat from agent to agent.

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CENTRAL NEURONAL PATHWAYS AND THE PROCESS OF ANAESTHESIA

A. ANGEL

The effects of anaesthetic agents are complex. When administered to humans they produce: (a) loss of conscious experience and a decrease in the amount of information which reaches the cerebral mantle, as measured by evoked responses to sensory stimulation; (b) loss of memory; (c) changes in the spontaneous activity recorded from the scalp (electroencephalogram (EEG)) or directly from the cortical surface (electrocorticogram (ECoG)); (d) loss of volitional control of the skeletal musculature; (e) an attenuation of protective reflexes (reflex withdrawal, cough and gag reflexes); (f) loss of postural reflexes; and (g) wide ranging changes in cardiovascular and respiratory performance.

As an experimental tool, anaesthesia may be designed, by appropriate choice of anaesthetic, to either enhance, suppress or leave unchanged various physiological variables which one wishes to study. For example, presynaptic inhibition may be enhanced or suppressed using barbiturates and urethane, respectively [13] and complex polysensory heterosegmental reflexes may be retained in animals anaesthetized with chloralose.

The usual structure–activity relationship seen with most bioactive chemicals is absent in anaesthetics. There is a wide range in molecular structures, ranging from simple chemicals, such as nitrous oxide, to the complex steroids, such as alphaxalone which may, under appropriate conditions, lead to a state of anaesthesia. Although in an interpretational and behavioural sense, anaesthetics may be antagonized by for example, convulsant chemicals, the only effective antagonist is very great ambient pressure. The antagonism of anaesthesia by pressure is not an antagonistic effect of competition with a receptor site by a specific blocking chemical, but a physico-chemical barrier to an anaesthetic, interacting with a putative hydrophobic receptor site. Looked at simplistically, the interaction between an anaesthetic and its putative site of action causes an increase in volume; increasing the ambient pressure prevents this volume increase.

For the study of anaesthesia, various simplifications need to be made, the first of which is the theory that because of the similarity of the end-point

of the state of anaesthesia, anaesthetics may share a common mechanism of action. The contrary view would state that each anaesthetic agent has a unique action, the end result of which is to produce a state of anaesthesia. However, the simplicity of the idea of a common mode of action is much more appealing, and to this end I will attempt to address the question “Do anaesthetic agents have a similar action which may be used to explain one or more facets of their activity?” This question will be looked at, not from a simple chemical interaction of anaesthetic agents with a potential receptor, but more globally to determine if there are anaesthetic sensitive parts of the central nervous system which, if perturbed, may set in train an integrated series of events which may explain one or more of the facets observed in clinical practice. In order to achieve this, it is necessary to look first at anatomy and then attempt to explain functional observations.

ANATOMICAL OBSERVATIONS

One region of the central nervous system (CNS) which has been the target of much research in attempting to define consciousness/arousal, waking/sleeping or attentional switching, is the brain stem reticular formation. This part of the nervous system is depicted in textbooks usually as a sausage-like structure, occupying the central portion of the medulla, pons and midbrain, connected by arrows to every part of the brain and spinal cord, and receiving an afferent input from all sensory modalities. Although this is a somewhat simplistic representation, it reflects the importance and generalized activity of the structure, but may be misleading. The first indication that this part of the CNS may be involved in the generation of the anaesthetic state was inferred by Magoun and his collaborators [19, 22] from its role in co-ordinating sensory and motor mechanisms related to the level of behavioural and electrocortical arousal of animals. They showed that the brain stem reticular formation controlled the excitability of spinal motoneurons [16, 22], and that high frequency electrical stimulation within the reticular complex gave rise to generalized electrocortical arousal, while low frequency stimulation produced generalized electrocortical synchronization [21].

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KEY WORDS

Anaesthesia: brain stem reticular formation, central nervous system, electroencephalogram, mode of action.

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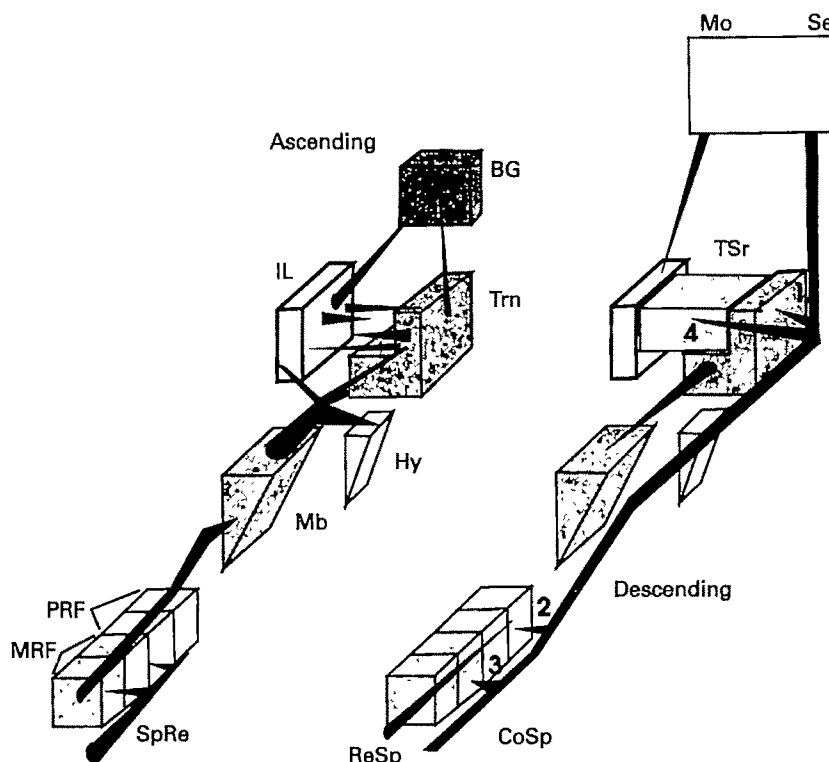


FIG. 1. Schematic diagrams of the major component of the brain stem reticular formation and its ascending and descending projections. The right hand side of a quasi-three-dimensional straightened brain has been represented twice to show the projections. For clarity, the thalamic sensory relay block has been left out of the ascending projection diagram. BG = Basal ganglia; CoSp = corticospinal projection; Hy = hypothalamus; IL = intralaminar nuclei; Mb = mesencephalon; Mo = motor cortex; MRF = medullary reticular formation; PRF = pontine reticular formation; ReSp = reticulospinal projection; Se = sensory cortex; SpRe = Spinoreticular projection; Trn = thalamic sensory relay nuclei. 1, 2 and 3 = Cortico-reticular projections; 4 = cortico-thalamic projection.

The brain stem is commonly taken to include the medulla, pons and mesencephalon. The reticular formation is a complex of discrete cellular regions in the medial part of the brain stem, which are anatomically less clearcut than those cellular aggregations associated with the cranial nerves and dorsal columns [9]. The medullary and pontine part of the brain stem reticular formation is composed of a ventrolateral portion projecting to the cerebellum and a medial portion sending long afferent projections to higher and lower structures. This medial part may be subdivided further into four separate areas: (a) a "major" area comprising a medullary component, the nucleus reticularis gigantocellularis, nucleus reticularis ventralis and nucleus reticularis lateralis, and a pontine component, the nucleus reticularis pontis oralis and nucleus reticularis pontis caudalis; (b) a noradrenergic projection from the pontine locus coeruleus nuclei; (c) a 5-hydroxy-tryptaminergic projection from the midline raphe nuclei in the medulla and pons; and (d) a cholinergic projection from the pedunculo-pontine and latero-dorsal tegmental nuclei.

The major component (fig. 1)

Cells in the medial parts of the medullopontine compartment of the system show extensive discord dendritic arborization, aligned transversely, as are the terminal arborizations of afferent fibres terminating in this region. It receives afferent fibres from the spinal cord (spinoreticular tract), from

acoustic, vestibular, trigeminal, spinothalamic and vagal sources as afferent collaterals and from the visual system via tecto-reticular fibres. Three types of cells are found; those with long ascending axons, long descending axons or both. Although segregation between cells with long ascending or long descending axons cannot be made, most of the cells with long descending axons (reticulospinal) lie in the middle portion of the pons (projecting ipsilaterally) and in the rostral part of the medulla (projecting bilaterally to the spinal cord). In contrast, cells with long ascending axons are found in the caudal part of the pons (caudal half of the nucleus reticularis pontis caudalis) and the caudal part of the medulla (caudal half of the nucleus reticularis gigantocellularis, nucleus reticularis ventralis and nucleus reticularis lateralis).

Spinoreticular fibres terminate mainly in those areas giving rise to long ascending axons and cortico-reticular fibres end in those parts giving rise to long descending axons. One can describe therefore, a system receiving a polysensory afferent input and projecting to the midbrain; and a descending system with an input from the cerebral cortex, at this level mainly from the motor cortex, and projecting to the spinal cord. These two systems are not distinct but functionally and anatomically interrelated, since part of the cellular component gives rise to both long ascending and long descending axons.

Those areas which are involved in respiratory, cardiovascular, visceral and skeletomotor control

show a close coincidence with the reticular areas giving rise to long descending axons. Whether or not this reflects an intermingling of numerous functionally dissimilar neurones or groups of functionally dissimilar neurones with reciprocal connections, remains unclear. However, it may be considered as a site for the integration of visceral-skeleto motor acts of obvious advantage in "arousing" or sleeping reactions. For example, leaping out of bed by activation of the skeleto-motor system without preparative changes to cardiovascular and respiratory effort would be both dramatic and disastrous!

The upward projection of the medullopontine reticular formation is mainly to the mesencephalon—to a dorsal zone in the nucleus cuneiformis and a ventral zone in the nucleus sub-cuneiformis. The other projection is to the hypothalamus and the ventral part of the thalamic reticular nucleus. The major upward projection from the mesencephalon (approximately 75% of fibres), goes to subthalamic sites—to the zona incerta and hypothalamus. The remainder project to the thalamic reticular nucleus and the intralaminar nuclei.

The intralaminar nuclei may be separated into a rostral portion comprised of the central medial, paracentral and central lateral nuclei and a caudal portion comprised of the centrum medianum-parafascicular (CMP) complex. The intralaminar nuclei project densely to the basal ganglia and cells located in CMP complex project to every other thalamic nucleus, especially to the cerebello-thalamic relay nuclei (nuclei ventralis lateralis and ventralis lateralis) and to the thalamic reticular nucleus. The intralaminar nuclei receive a topographically organized projection from the motor cortex.

The efferent projection from the thalamic reticular nucleus is directed to three sites: (i) the mesencephalic tegmentum, (ii) the specific thalamic relay nuclei for the somatosensory, auditory and visual pathways and (iii) the intralaminar nuclei. Of special interest is the observation that the thalamic regions which receive a projection from the thalamic reticular nucleus project back into this nucleus [24]. The thalamic reticular nucleus also receives an orderly and topographically arranged cortical projection from all of the major cortical primary sensory receiving areas [10].

One further anatomical feature of note is the interrelation between the thalamic relay site receiving a reticular projection, the cortical target of the relay site and the cortical back projection to both the thalamic relay and reticular sites. This is organized as follows: (i) a specific site in the thalamic reticular nucleus projects to a specific thalamic relay site (*reticulo-thalamic*); (ii) this thalamic relay site projects to a specific cortical site (*thalamo-cortical*); and (iii) this cortical site projects back to both the thalamic relay site (*cortico-thalamic*) and to the thalamic reticular site (*cortico-reticular*) projecting to that thalamic relay site.

Thus there is a precisely arranged neural loop of reticular-thalamic, thalamo-cortical, cortico-thalamic and cortico-reticular axons. In this loop the thalamic relay cells are exposed to reticular, cortical

5-HT

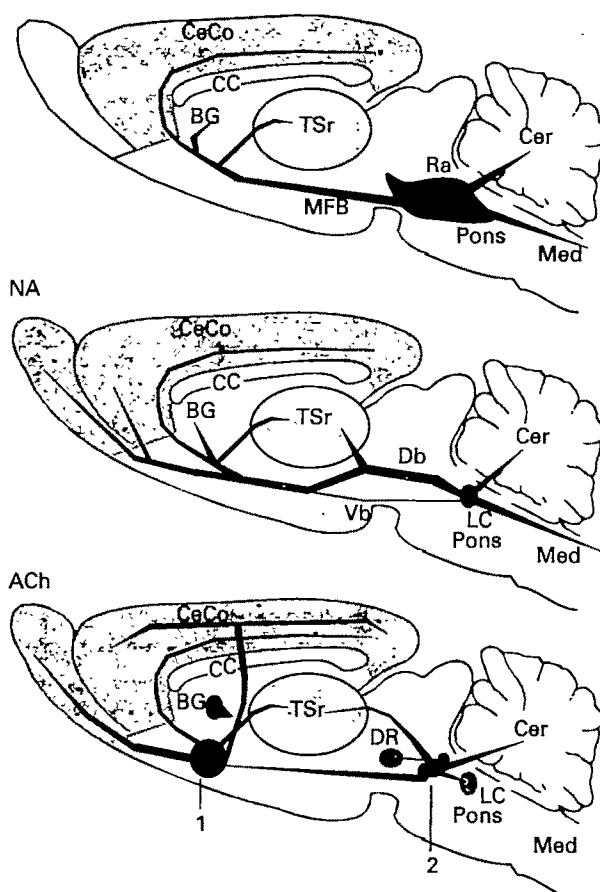


FIG. 2. Schematic diagrams of the 5-hydroxytryptaminergic (5-HT), noradrenergic (NA) and the cholinergic (ACh) projections, in the rat, viewed in the sagittal plane. BG = Basal ganglia; CC = corpus callosum; CeCo = cerebral cortex; Cer = cerebellum; Db = dorsal noradrenergic bundle; LC = locus coeruleus; Med = medulla; MFB = medial forebrain bundle; Pons = pons; Ra = raphe nuclei; TSr = thalamic sensory relay nuclei; Vb = ventral noradrenergic bundle. 1 = Basal forebrain cholinergic system (medial septal nucleus, diagonal band nuclei, substantia innominata, magnocellular preoptic field, nucleus basalis); 2 = upper brain stem cholinergic system (laterodorsal tegmental nuclei, pedunculopontine nuclei).

and cortico-reticular inputs. The thalamic extension of the brain stem reticular formation and the intralaminar and thalamic reticular nuclei, possess an anatomical arrangement which would modify the activity of thalamic nuclei projecting onto the cortical mantle and the basal ganglia, which could serve to produce severe sensory and motor disturbances which would then be amplified by the brain stem reticular control of spinal motoneurons.

Noradrenergic projection (fig. 2)

Other structures which may alter the excitability of the nervous system in a non-specific way are the locus coeruleus nuclei located in the rostral pons. Cells in these nuclei, which comprise approximately 50% of all noradrenergic cells in the brain stem, give rise to long ascending and descending axons. The ascending axons from the coerulear cells ascend mainly ipsilaterally in the dorsal tegmental bundle (the majority), and in the central tegmental tract, to innervate the dorsal thalamus, the hypothalamus, the

cerebellum, the basal forebrain and the neocortex. Fibres in the cerebral cortex travel along the corpus callosum and send radial branches in the cortex to form a T junction with extensive ramifications in cortical layer I. The descending fibres pass to the spinal cord.

The mode of termination of the axons is different from conventional synaptic contacts. Each nerve axon may give rise to a long terminal zone with several thousand expanded portions called nerve terminal varicosities. Thus activation of noradrenergic neurones will release noradrenaline over a wide area, compared with the restricted terminal bouton release from other terminating axons. The remainder of the noradrenergic neurones are distributed diffusely throughout the ventral lateral tegmentum. Their ascending axons innervate the same structures as the coerulean axons, although they only provide a minor input to the cerebral cortex compared with that of coerulean cells. The locus coeruleus receives an afferent input only from two brain stem sites; the nucleus paragigantocellularis and the nucleus hypoglossi prepositus.

5-Hydroxytryptaminergic projection (fig. 2)

Another system with longitudinally aligned efferents is seen in the midline raphe nuclei. The nucleus raphe magnus receives afferents from the spinal cord and cerebral cortex and is also the principal site of origin of descending efferent fibres. Long ascending fibres originate from all nuclear groups in the raphe but the principal sites arise from the more rostral parts; the nuclei raphe dorsalis, raphe medianus and centralis superior. These ascending fibres innervate the thalamus and all parts of the cerebral and cerebellar cortices. The descending projections modulate the activity of spinal sensory and motor neurones. The 5-hydroxytryptaminergic innervations to the dorsal horn of the spinal cord depress afferent nociceptive input.

Cholinergic projection (fig. 2)

Two major constellations of nuclear groups have been found with long efferent axons. The first is in the basal forebrain cholinergic complex (medial septal nucleus, diagonal band nuclei, substantia innominata, magnocellular preoptic field and nucleus basalis) projecting to all parts of the cerebral cortex and olfactory bulb and the second is the ponto-mesencephalo-tegmental cholinergic complex (laterodorsal tegmental nuclei and pedunculo-pontine nuclei) which projects to the thalamus, the dorsal raphe nucleus, the locus coeruleus, the pontine and medullary components of the reticular formation and to the spinal cord.

PHYSIOLOGICAL OBSERVATIONS

One aspect of anaesthesia is that humans become unaware of stimuli from their external environment which are continually activating the peripheral sensory structures. The sensory pathways are organized such that the sensory transducers send their afferent axons into separate nerve tracts (e.g. somato-sensory information from the neck, trunk and limbs

are conveyed in the reticulospinal, spinothalamic tracts and the dorsal columns and visual information into the cellular layers of the retinae and hence into the optic nerves). The information in the afferent pathways used for the interpretation of the events in the immediate (touch, thermal sensations and taste) or the remote (sight, hearing and smell) environment is conveyed to special parts of the cerebral cortices; either the primary cortical receiving areas or, in the case of olfactory stimuli, to the olfactory bulb for initial processing. The sensory surfaces are mapped onto the cerebral cortex to produce distorted maps.

For example, the sensory homunculus is found along the postcentral gyrus and the retina is represented in the occipital cortex. This organizational wiring of the nervous system may be used to detect deliberate activation of any sensory input since if a particular part of any sensory surface is stimulated a particular population of cortical cells is activated. The current flow consequent upon this activation may be detected, amplified and displayed and is seen as evoked cortical responses. Because this deliberately activated current flow is superimposed upon the spontaneous electrical activity of the cerebral cortex, these individual responses are sometimes difficult to detect. However, if a series of stimuli are delivered to the same peripheral site, it is possible to extract the averaged evoked cortical response.

If the total current flow is summed with respect to the time of application of each stimulus, then stimulus related currents will add in direct proportion to the number of stimuli delivered, whereas the random (spontaneous) current flow will be summed in proportion to the square root of the number of responses. If the stimulus evoked response is regarded as the signal and the spontaneous current as noise, then after, for example, 100 summations the signal:noise ratio will become 10:1, or the noise will contribute to only 10% of the average. The recording of averaged responses provides the ability to assess sensory performance in a quantitative manner without recourse to asking a sentient subject what he feels; of obvious importance in the study of anaesthesia which has a literal translation of without feeling. The technique may moreover be applied to animals.

In all animals, including humans, the averaged responses recorded from the primary cortical receiving areas in response to electrical stimulation of peripheral nerves, or flashes or clicks, appear as a wave of surface positivity superimposed upon which are a series of negative waves or inflections. Typical evoked responses recorded from rats are shown in figure 3, illustrating the initial positive and negative waves and second positive and negative waves (labelled P_i , N_i , P_s , N_s , respectively). The rat is commonly used because it has an unfolded cortical surface (lissencephalic) which makes the recording geometry and interpretation of the response easier than in animals with folded cortices. In order to understand the results presented in this section, it is necessary, although not essential, to document the techniques used to obtain the results (see Appendix).

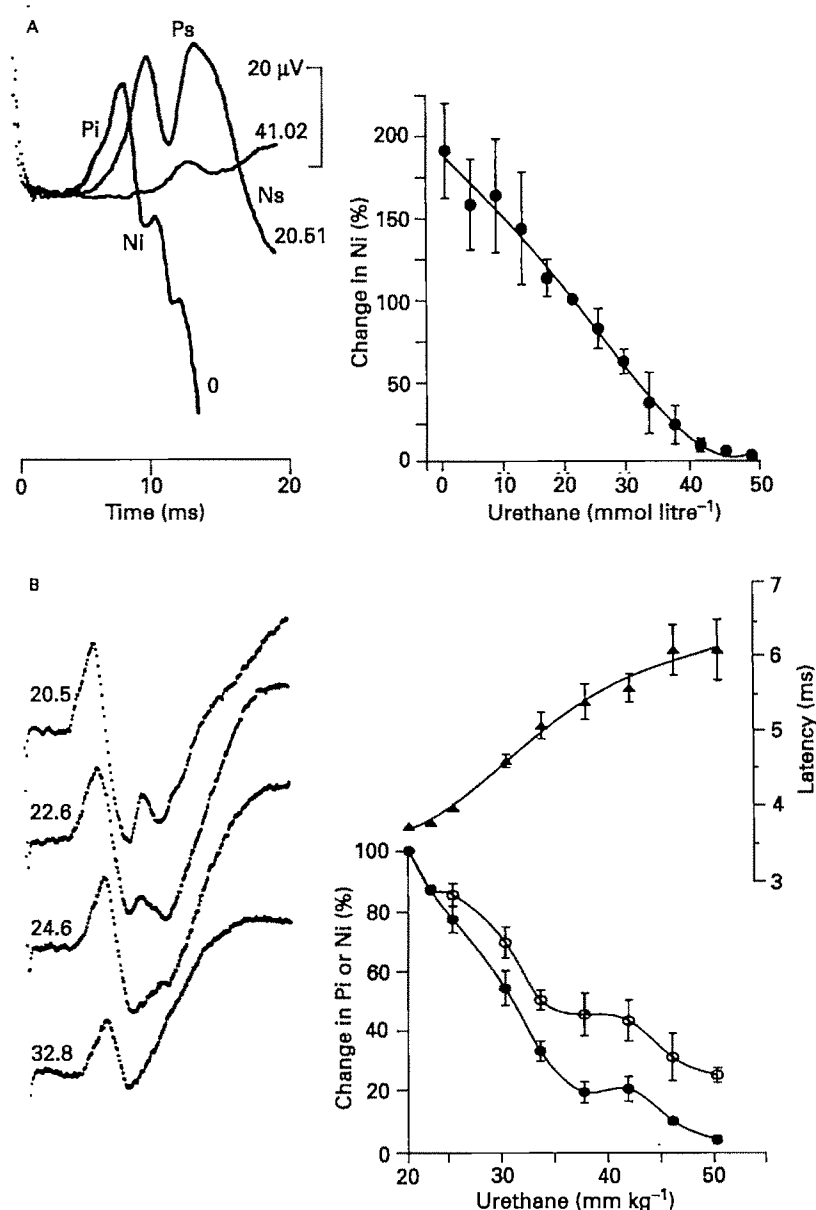


FIG. 3. A: Three superimposed averaged evoked cortical responses to electrical stimulation of the contralateral forepaw at a rate of 1 s^{-1} . Each average is 128 consecutive responses in the unanaesthetized (0), the surgically anaesthetized (urethane $20.51 \text{ mmol litre}^{-1}$) and the very deeply anaesthetized ($41.02 \text{ mmol litre}^{-1}$) animal. For this series of responses the animal was pretreated with chlordiazepoxide (15 mg kg^{-1}) to obtain a stable condition in the unanaesthetized state. The percentage change in Ni from four animals *vs* anaesthetic dose is shown to the right. The responses have been scaled such that 100% equals the concentration found in the surgically anaesthetized animal ($20.51 \text{ mmol litre}^{-1}$). Data are mean, SEM. B: Averaged evoked responses from one animal (128 consecutive responses to electrical stimulation of the contralateral forepaw at a rate of 1 s^{-1}) starting at a surgical concentration of urethane anaesthesia (mmol litre^{-1}) and adding urethane. The dose-response curves from 12 animals are shown on the right. The ordinates for Pi (○) and Ni (●) have been scaled such that 100% equals the response level at surgical anaesthesia, the abscissa shows the theoretical concentration of urethane in the aqueous phase. Data are mean, SEM.

The response curve for Ni is not significantly different from that for the four animals shown in (A).

GROUP A ANAESTHETICS

In the unanaesthetized animal, the early part of the evoked cortical response is predominantly negative, showing definite Pi and Ni waves, but with the second positive wave seen only as a small inflection between Ni and Ns (see fig. 3A). Comparing the latencies to the start of the evoked cortical responses in a series of experiments shows that the latency increased from 3.50 (SEM 0.08) ms ($n=9$) in unanaesthetized animals to 4.20 (0.05) ms ($n=86$) at

the usual starting level of urethane anaesthesia of $20.51 \text{ mmol litre}^{-1}$ (this is the theoretical concentration of urethane if the dose administered is freely distributed throughout the aqueous compartment of the rat and represents an anaesthetic dose of 1.25 g kg^{-1}), a highly statistically significant increase (Student's *t* test two-tailed, $P < 0.001$). A further increase in urethane anaesthesia to $41.02 \text{ mmol litre}^{-1}$ gave a further increase in latency to 5.90 (0.25) ms ($n=12$), which was also a statistically significant increase ($P < 0.001$). As the

TABLE I. The groups of anaesthetics and the effects seen upon sensory transmission for each anaesthetic of that group. Inc. = Increase, Dec. = decrease, Inc./d. = increase in probability of response but a large increase in the dispersion of the discharge. — = Information is not yet available for that group. Increases or decreases relate to amplitude for Pi and Ni and probability of response for VPL, III, IV, V and VI (for further details see text)

Anaesthetic	VPL	Latency	Pi	Ni	III	IV	V	VI
Group A								
Cyclopropane	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Nitrous oxide	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Diethyl ether	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Enflurane	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Halothane	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Isoflurane	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Methoxyflurane	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Trichloroethylene	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Vinylethyl ether	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Althesin	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Ethyl carbamate	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Inactin	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Ketamine	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Hexobarbitone	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Methohexitone	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Pentobarbitone	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Thiopentone	Dec.	Inc.	Dec.	Dec.	Dec.	None	Dec.	Little
Group B								
Chlordiazepoxide	Inc.	Inc.	Inc.	Dec.	Dec.	Dec.	Dec.	Dec.
Midazolam	Inc.	Inc.	Inc.	Dec.	Dec.	Dec.	Dec.	Dec.
Etomidate	Inc.	Inc.	Inc.	Dec.	Dec.	Dec.	Dec.	Dec.
Propofol	Inc.	Inc.	Inc.	Dec.	Dec.	Dec.	Dec.	Dec.
Noradrenergic group								
Clonidine	Inc./d.	Inc.	Dec.	Dec.	—	Inc./d.	Inc./d.	
Medetomidine	Inc./d.	Inc.	Dec.	Dec.	—	Inc./d.	Inc./d.	

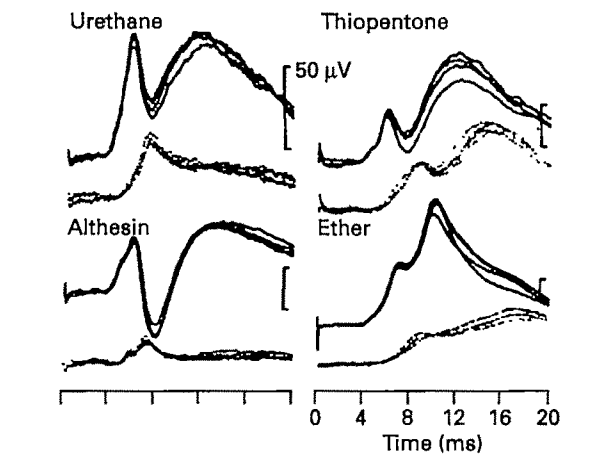


FIG. 4. Four averages superimposed ($n = 64$) before (continuous lines) and after (dotted lines) dose of anaesthetic is added. The anaesthetics were approximately equipotent. The calibration bars represent $50\text{ }\mu\text{V}$ for each pair of records. Note the extreme variability of the responses obtained at the starting concentration of anaesthesia by comparing the shapes and sizes of all continuous line records (from [2] with permission).

latencies of the responses increased, the amplitudes of the initial positive and negative waves of the cortical response decreased. Figure 3A shows that there is a dose-response continuum relating the depth of anaesthesia to the total change in the cortical evoked response when starting from the unanaesthetized animal and proceeding to very deep levels of anaesthesia, or when the anaesthetic is superimposed upon a basal level of urethane (see fig. 3B).

Most anaesthetics that are used in a clinical context to produce prolonged general anaesthesia (see table

I, fig. 4) have been found to increase the latency and decrease the amplitudes of the initial components of the cortical response, either when starting with the animal unanaesthetized [3] or when superimposed upon the initial level of urethane anaesthesia [5]. Although nitrous oxide behaved in the same way as the other anaesthetic agents, its effect on response latency and Pi was always found to be transient, the cortical response quickly returning ($18.9\text{ (}2.35\text{ min, }n = 15\text{)}$) to pre-administration levels even during prolonged periods (up to 90 min) of nitrous oxide administration (see [5]). It must be remembered, however, that the anaesthetic dose of nitrous oxide is obtained only at a partial pressure of 141 kPa.

Four exceptions were found. Neither propofol nor etomidate gave the same large changes in the evoked cortical response when superimposed upon a background of urethane anaesthesia (see below). The others were the benzodiazepines chlordiazepoxide and midazolam (see table I, group B).

The cerebral evoked response in animals deeply anaesthetized with urethane ($41.02\text{ mmol litre}^{-1}$) subjected to great ambient pressure shows, as the pressure is increased with helium (in an environment with a constant partial pressure of oxygen of 55 kPa), a decrease in the latency of the cortical response, and an increase in the amplitudes of the initial components of the response are seen [6]. For example, in one experiment, the initial negative wave was 100% (SD 9%) at the start and increased to 202% (16%) and 328% (16%) at pressures of 6868 and 10000 kPa, respectively. Control experiments run in the pressure chamber over the same time scale (approximately 2 h) as the pressure experiments, but without changing the pressure, showed that this effect could

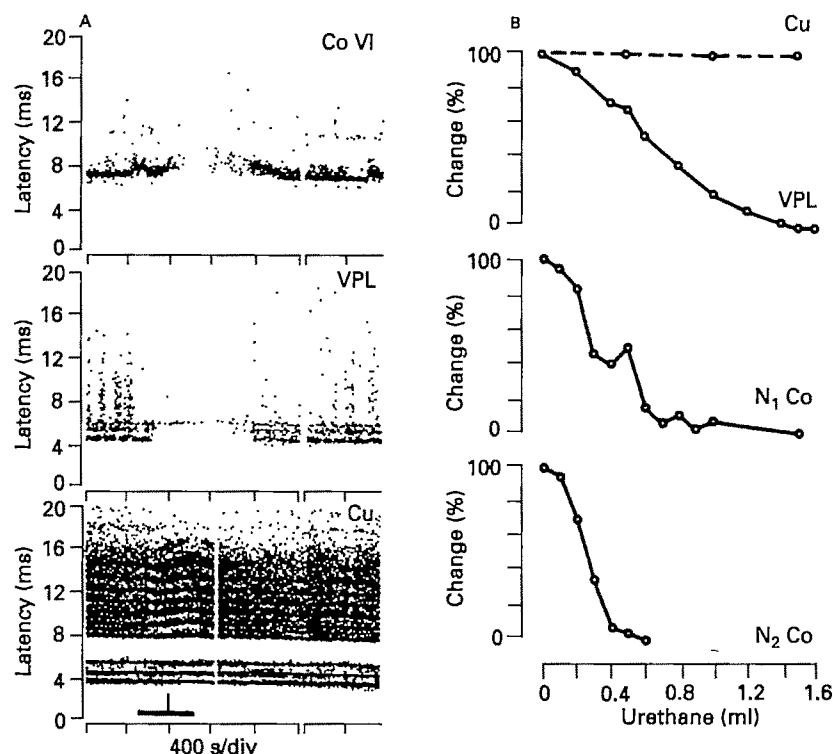


FIG. 5. A: Responses obtained from three cells (the cuneate nucleus (Cu), the ventrobasal thalamus (VPL) and from cortical layer VI (Co VI)) isolated from sites along the somatosensory pathway from one animal anaesthetized with urethane. Each block shows a 47 min record with 3% isoflurane (I) in oxygen administered for 500 s, beginning 500 s after the start of the record. The forepaw was stimulated at a rate of 1 s^{-1} . B: Mean change in probability of response plotted as percent starting concentration *vs* depth of urethane anaesthesia in ml of anaesthetic added. The starting concentration of urethane anaesthesia was $20.51 \text{ mmol litre}^{-1}$ (the addition of 1 ml of urethane doubles this concentration). The responses are from 35 cuneate cells (Cu), 30 ventrobasal thalamic cells (VPL), 40 cortical cells isolated from layers IV and VI (N₁ Co), and four cortical cells isolated from layer V (N₂ Co) (from [2] with permission).

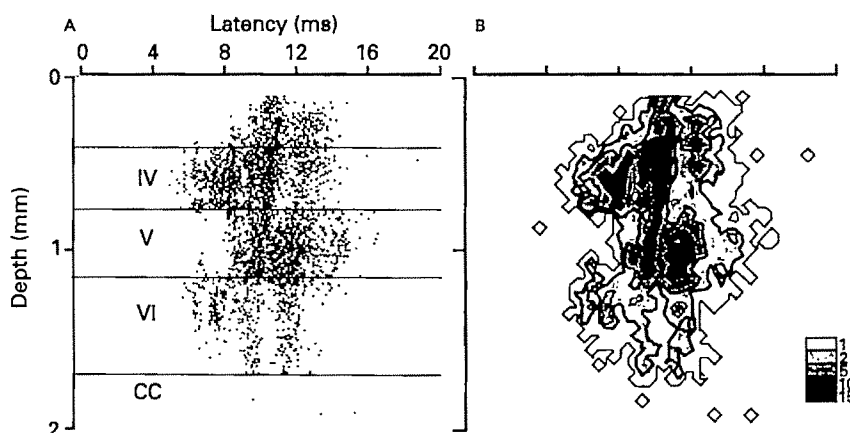


FIG. 6. A: (raw data) Response obtained by stepping a microelectrode through the cortex ($2 \mu\text{m}$ steps once every 1 s) and sampling the signal for cell discharges (dots) in response to electrical stimulation of the contralateral forepaw as latency of response *vs* depth. The approximate location of the cortical layers is shown as IV, V and VI, in addition to the position of the corpus callosum (CC). B: (contour plot) The raw data was considered as occupying a 50×50 matrix. The dot density in each square of the matrix was determined and lines of equal density constructed to map the response density *vs* depth.

not be a result of removal of the anaesthetic by metabolic activity as there were no changes in either the latency or the initial positive and negative waves of the cortical responses. Reducing the pressure in the chamber from 10100 to 6060 kPa gave the opposite effect (i.e. an increase in latency and a decrease in the amplitudes of the initial positive and initial negative waves); a response change similar to that of increasing the dose of anaesthetic. A similar

pressure reversal has been seen with Althesin, halothane and nitrous oxide [Angel & Halsey unpublished observations]. Thus the effect of anaesthetic agents on the evoked cortical response shows pressure reversal which is predicted by simple physicochemical theory. This has a corollary which may be interpreted to indicate that the anaesthetic effect is liable to be a true effect of anaesthesia and not an interesting side effect.

Thus the effect of a large group of anaesthetic agents is to change the evoked cortical responses which represent a change in the responses of cells at the cortical level. These changes may be interpreted in three ways: (a) the cortical cells respond differently to an unchanged ascending input; (b) the cortical cells are not affected but mirror a change occurring at a subcortical level; or (c) any combination of these two. Any effect on peripheral nerve axons or sensory transducers may be ruled out since nerve axons are affected only by lethal doses of anaesthetic [23], and there is no measurable effect of anaesthetics in rats over the ranges studied on the electroretinogram, cochlear microphonics or nerve volleys consequent upon mechanical stimulation of the integument.

The pathway which conveys information to the cortex, with a short latency, in the rat has been shown to be the dorsal column–lemniscothalamic path [2]. Thus the afferent signal has to cross only two synaptic discontinuities before it reaches the cerebral cortex. The first of these is in the dorsal column nuclei and the second in the ventrobasal thalamus. The ability of anaesthetic agents to influence cells at these relay sites may be assessed by recording the extracellular current flow which is seen after afferent stimulation. Cells in the dorsal column nuclei are unaffected by any of the anaesthetic agents listed in table I until near fatal levels of anaesthesia are reached. The changes seen are, paradoxically, to stabilize the responses of the cells; particularly those which respond to a single stimulus with a prolonged discharge (see fig. 5A). Cells in the ventrobasal thalamus do not respond to every peripheral stimulus; even though the stimulation levels are supra-maximal for dorsal column nuclear cells. Cells in the cuneate nucleus show a response probability of unity (i.e. they respond to each peripheral stimulus). Cells in the ventrobasal thalamus respond with an average probability of approximately 0.8 (i.e. they respond, on average, to four out of every five stimuli presented). The ventrobasal thalamus therefore represents a site of synaptic insecurity. The effect of increasing anaesthesia (with the anaesthetics listed in table I, group A) on ventrobasal thalamic cells was always the same: to increase the latency of discharge and to decrease the probability of response in a dose-dependent manner (see fig. 5A). Cells in the cerebral cortex thus show a decreased response and increase in latency, partly because of the diminished thalamocortical volley (see fig. 5A). The average responses to increasing anaesthetic depth of cuneate and ventrobasal thalamic cells are shown in figure 5B.

Since the cortex is a layered structure, with each layer having a different function, for a proper study of cortical neurones it is necessary to study the behaviour of cells in each layer. A depth–latency of response technique has been devised, in which a microelectrode is lowered through the cerebral cortex at the geometric centre of the primary somatosensory perpendicular to its surface, starting at the pial surface, in small discrete steps. At each vertical position an electrical stimulus is delivered to a peripheral nerve and the microelectrode signal is processed to detect whether or not cellular dis-

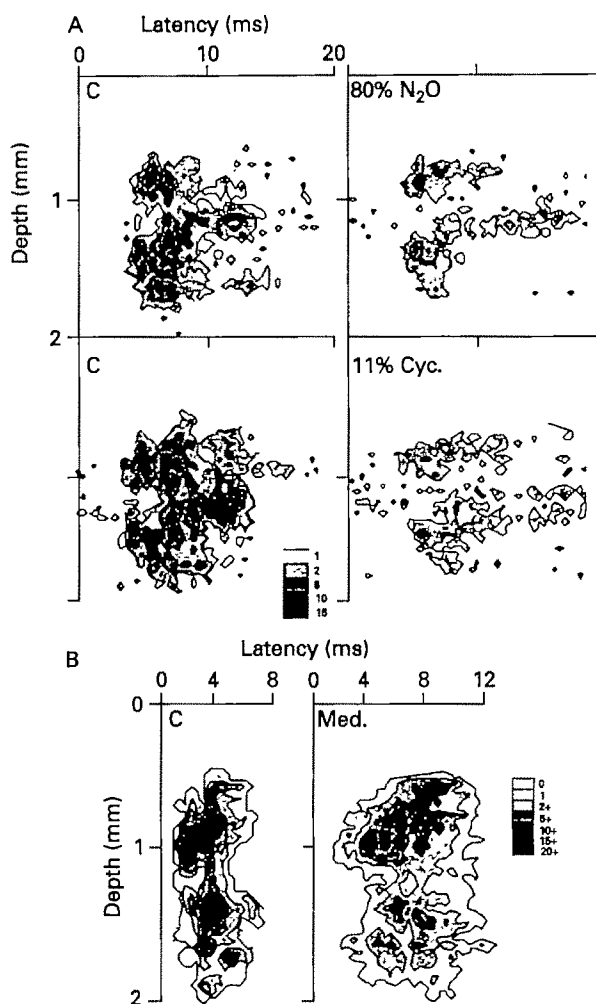


FIG. 7. A: Contour plots from a rat anaesthetized with urethane 20.51 mmol litre⁻¹ in two control (C) conditions (left hand side) and at the maximum effect of two equipotent doses (0.5 MAC) of nitrous oxide (N₂O) (80%) and cyclopropane (Cyc.) (11%). All records were obtained from one animal and a 2-h period was given between the end of nitrous oxide administration and the second control plot. B: Similar plots from a rat anaesthetized with urethane 20.51 mmol litre⁻¹ before (control (C)) (left hand side) and after medetomidine (Med.) 100 µg kg⁻¹ i.p.

charge(s) occur. Nerve cells respond to a stimulus with an all-or-nothing nerve action potential. Thus the signal is passed to a voltage level detector which gives out a standard pulse each time the microelectrode signal exceeds three times the noise (or baseline) level. Such signals are found to be associated with nerve cell discharge. These pulses are stored in a microprocessor with references to the stimulus occurrence (i.e. their latency is recorded) and to the vertical position of the microelectrode. In each second the microelectrode is lowered 2 µm and a stimulus is delivered to a peripheral nerve. After 1000 such steps, the cortex will have been penetrated from the pial surface to the corpus callosum. A record is then constructed, in the microprocessor, of nerve cell discharge occurrence latency (as a dot) *vs* depth. A typical record is shown in figure 6 (raw data). This record is then re-processed to display dot density as a contour map (see fig. 6). Both graphs show that cellular discharges with an early latency occur in two bands (from approximately 400–800 µm and 1200–1600 µm below the surface) separated by a

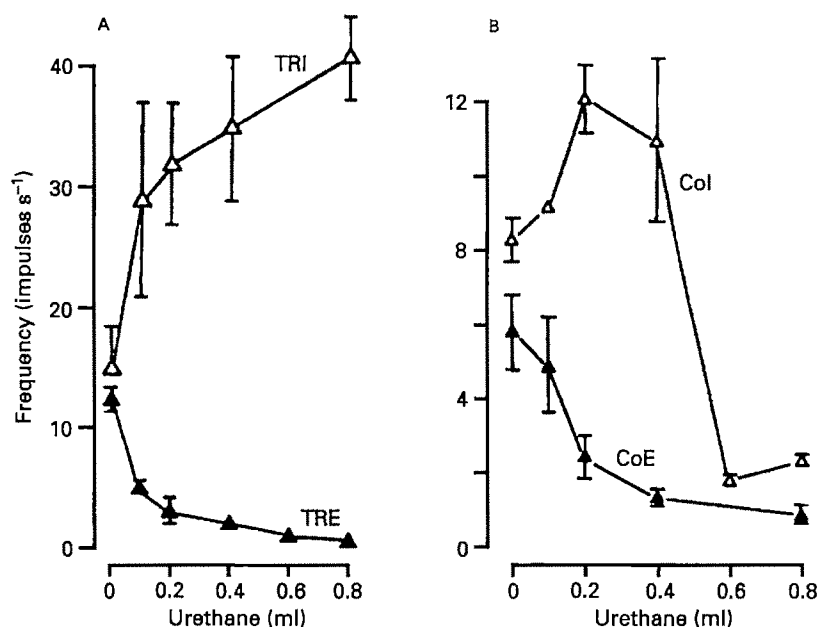


FIG. 8. A: Mean change in frequency of discharge of six thalamic reticular/boundary cells of both types (TRE and TRI) expressed as impulses per second *vs* increasing depth of urethane anaesthetic. B: Similar frequency *vs* anaesthetic plot for eight cortical presumed excitatory cells (CoE) and four cortical presumed inhibitory cells (CoI). Data are mean, SD (from [2] with permission).

band of later cellular responses. These bands correlate very closely to the anatomical boundaries of cortical layers IV and VI, and layer V, respectively. The contour map shows moreover that the major latency densities occur at 8 and 11 ms in layer IV, 10 and 12 ms in layer V and 8 ms in layer VI. By applying this technique before and after anaesthetic administration, responses such as those shown in figure 7A are seen. Note that in figure 7, which shows the effect of added equipotent doses of nitrous oxide and cyclopropane on a basal concentration of urethane anaesthesia, the depth latency responses are both shifted to the right (an increase in overall latency of response) and the "hills" described by the contour map are lower (an overall decrease in responsiveness). More noteworthy is the observation that the responses in the middle layer (layer V) are effected more by the increase in anaesthetic concentration than the cells in layers IV and VI. Additionally, cells in layer III also show increased susceptibility to anaesthesia. This pattern of response is seen with all the anaesthetic agents described as group A in table I. Comparison of the effects of anaesthetic increases on single cells in layers IV and VI, and layer V is shown in figure 6. With all group A anaesthetics, cells in layers III and V show a much greater decrease in response to increasing anaesthetic depth than those in layers IV and VI. It may be shown that cells in layers IV and VI are themselves unaffected by group A anaesthetics, they merely mirror the decrease in the thalamocortical input. This may be shown since their responses are unaffected by increasing anaesthetic depth if they are activated by stimulation of thalamocortical fibres, either in the intact animal or in isolated cortical slices. In cortical slices, in comparison, layer V cells still show marked susceptibility to anaesthetic action (see [2]).

Therefore, two sites of synaptic transfer may be

described in the sensory pathway which are susceptible to the action of anaesthetics; in ventrobasal thalamus and the cortical layer V. Cells at other levels are unaffected (e.g. those in the dorsal column nuclei and cortical cells in layers IV and VI).

Recordings made from cells at the ventrobasal thalamic-thalamic reticular nucleus boundary, and in the thalamic reticular nucleus, show major differences in their activity from ventrobasal thalamic cells. First, thalamic reticular cells show spontaneous activity (i.e. activity in the absence of intentional stimulation) and second, they respond to potentially noxious stimulation (mechanical damage to the skin or heating to over 40 °C) applied over widespread areas of the skin (often the entire body surface). Thus they respond to potentially arousing stimuli. Two distinctive types of cell are encountered: those whose frequency of discharge is increased by "noxious" stimuli and those whose frequency is decreased by such stimuli. Since the central nervous system shows evidence of an increase in excitability after a potentially noxious stimulus, these two cell types may be interpreted as representing thalamic reticular excitatory (TRE) and thalamic reticular inhibitory (TRI) neurones, respectively (see [2]). Increasing depth of anaesthesia, using group A anaesthetics, always decreases the discharge frequency of thalamic excitatory cells and increases the discharge frequency of thalamic reticular inhibitory cells. Results from such cells showing the dose-dependent changes in cell frequency of discharge are seen in figure 8A.

There are two other groups of cells which are also implicated in the effect of anaesthetics. These are cells which show spontaneous activity located in cortical layer III (mainly at the III-IV boundary) and cells in layer V. These cells are of two major types, like the thalamic reticular cells, and behave as though they are either cortical inhibitory cells or

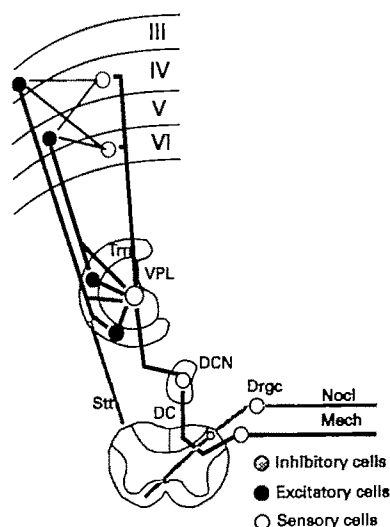


FIG. 9. Diagram of the neural paths involved in the genesis of the anaesthetized state (see text for details). III, IV, V and VI = cortical layers; DCN = dorsal column nuclei; Drgc = dorsal root ganglion cells; Mech = mechanoreceptive input; Noci = nociceptive input; Strt = spinothalamic and spinoreticular tracts; Trn = thalamic reticular nucleus; VPL = ventrobasal thalamus.

cortical excitatory cells. These cells are functionally in contact with the thalamic reticular cells such that cortical inhibitory cells excite thalamic reticular inhibitory cells and cortical excitatory cells excite thalamic reticular excitatory cells (see [2] for details). When subjected to increasing concentrations of anaesthetic, the cortical excitatory cells show a dose-dependent decrease in the frequency of discharge and for moderate increments of anaesthesia the cortical inhibitory cells show a dose-dependent increase in discharge frequency. However, at doses of urethane of approximately $30 \text{ mmol litre}^{-1}$, the electrocortical activity undergoes a spectacular change and cortical spindling activity occurs. This is at the dose at which the cortical inhibitory cells deviate from "expected" activity. One other aspect of these cortical cells is that approximately 50% are not only spontaneously active but also respond to mechanical stimulation of the skin.

Thus for group A anaesthetics, a hypothetical circuit may be drawn and is illustrated in figure 9. Any ventrobasal sensory relay cell will have its excitability modulated via three routes: (a) via a direct thalamic reticular nucleus input; (b) via a direct corticothalamic input; and (c) via a cortico-reticular thalamic input.

If the cortex is removed, then anaesthetics show little or no effect upon thalamic transmission of sensory information [2]. Thus it is the direct cortical and cortico-reticular thalamic modulations which are of importance. If anaesthetic agents were to have an action on cortical modulatory cells (layers III and V), this could cause more inhibition and less excitation of thalamic relay cells and hence a large decrease in the capacity of the thalamic relay nuclei to transfer information to the cerebral cortex. This could then be a self-reinforcing reaction, since some of the cortical modulatory cells are themselves sensitive to afferent information via the thalamic relay nuclei and the thalamus is subjected to cortical

influence via two routes: a direct cortico-thalamic input and an indirect cortical input via the thalamic reticular cells. In any case, a decrease in the afferent inflow to the cerebral cortex would mean a decrease in sensory experience.

GROUP B ANAESTHETICS

While the foregoing scheme can be applied to the vast majority of anaesthetics, some show exceptions to this behaviour. Among those which have received attention are propofol, etomidate and the benzodiazepines chlordiazepoxide and midazolam (see table I). These differ from group A anaesthetics in that they give a small increase in the amplitude of the initial positive wave of the cortical response but show the same increase in latency of response and decrease in the initial negative wave (see table I, fig. 10). The averaged responses illustrated in figure 10B show latencies of 3.58, 4.18 and 4.63 ms for control, propofol and methohexitone conditions, respectively. For these results, the propofol and methohexitone doses were chosen to be approximately equipotent. At the same time as these changes in latency were seen, the initial positive wave increased from 100 (sd 3.30)% to 110 (7.20)% with propofol but decreased to 90 (4.97)% with methohexitone. The initial negative waves were decreased by both anaesthetics from 100 (10.70)% to 40.11 (6.60)% and 38.59 (2.33)% for propofol and methohexitone, respectively. Since the amplitude of the initial positive wave may be taken as a rough index of the synaptic effectiveness of the thalamo-cortical volley [1] it is not surprising to find that, on average, the response probability of thalamic sensory cells is increased, which is coupled with increased latency. Figure 11A shows the average response probabilities of seven ventrobasal thalamic sensory relay cells which were recorded from long enough to compare the action of propofol with methohexitone. Note that after methohexitone, the latency of response shows a comparable increase with that after propofol, but that in contrast to the action of propofol, the response probability is decreased. This decreased thalamo-cortical volley is reflected in the decreased initial positive wave of the evoked cortical response (compare fig. 10B records P and M). Another major difference seen with propofol is in the responsiveness of cortical cells to afferent information. Unlike group A anaesthetics, the transfer of this information is impeded in all cortical layers. Cells in cortical layer IV do not show an increase in the probability of discharge which they should do if they were to mirror the ventrobasal thalamic volley, as they do with group A anaesthetics, and cells in cortical layers II, V and VI all show the same apparent susceptibility (see fig. 11B). As with all group A anaesthetics, group B anaesthetics do not affect the transfer of information at the first sensory relay, in the dorsal column nuclei. Thus the group B anaesthetics disrupt the transfer of information through the ventrobasal thalamus to give a net increase in the signal transmitted from this nucleus to the cerebral cortex. The action of propofol on ventrobasal thalamic cells is complex. Of the 17 cells

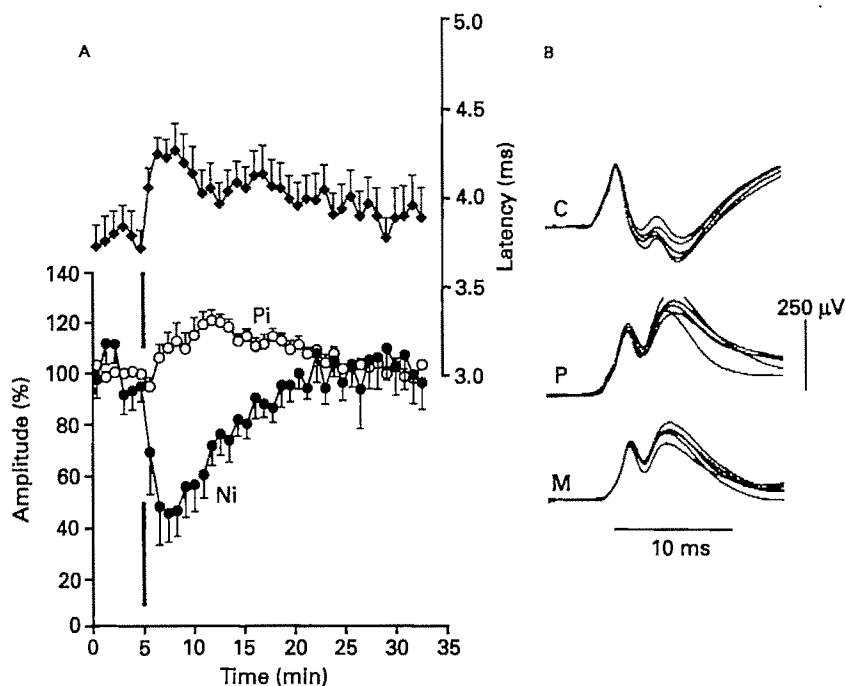


FIG. 10. A: Average time course of action of an i.v. injection of propofol 5 mg kg^{-1} (vertical line) in 25 rats surgically anaesthetized with urethane. Data are mean, SEM. B: Five averaged cortical evoked responses superimposed from a rat surgically anaesthetized with urethane (each average of 50 consecutive stimuli applied to the contralateral forepaw at a rate of 1 s^{-1}) before (control (C)) (upper traces) and at the peak of the effect of an i.v. dose of propofol (P) 5 mg kg^{-1} . M = Methohexitone.

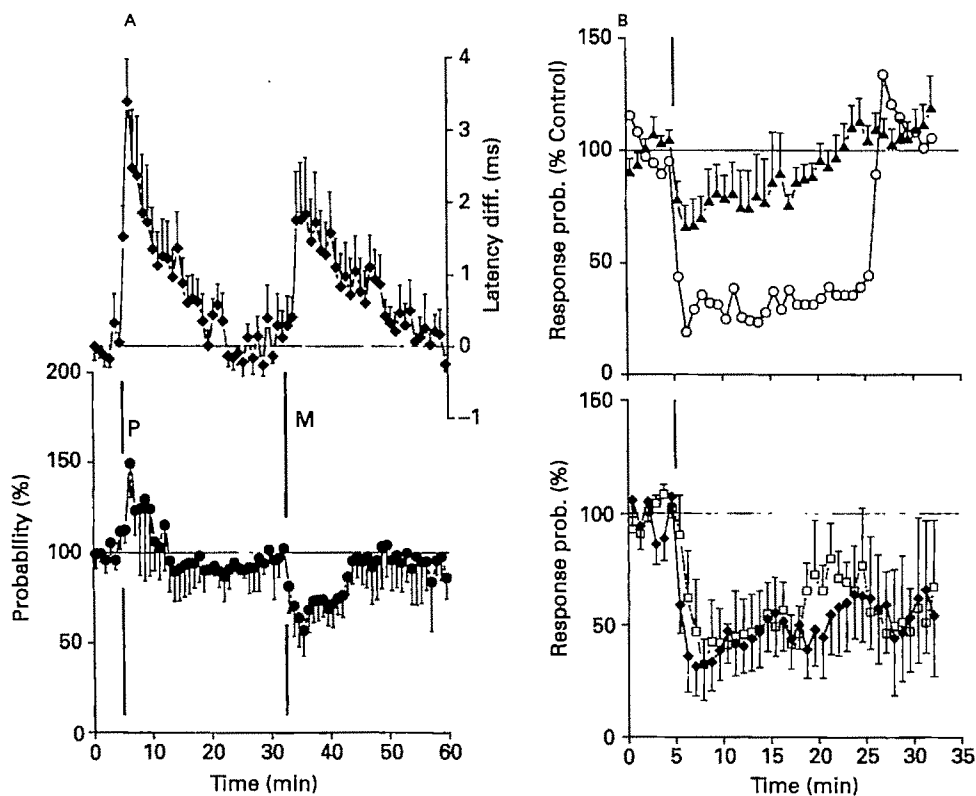


FIG. 11. A: Effect of approximately equipotent doses of propofol (P) 5 mg kg^{-1} i.v. and methohexitone (M) 5 mg kg^{-1} i.v. on the response of seven cells isolated from the ventrobasal thalamus in rats anaesthetized with urethane. The upper graph shows the difference in the median latency of response from the average median latency in the period prior to propofol administration. The lower graph shows the probability of response. The response for each cell was normalized to 100% for the period prior to propofol administration. Data are mean, SEM. B: Effect of i.v. propofol 5 mg kg^{-1} on a single cell from cortical layer III (○) and 12 cells from cortical layer IV (▲) (upper graph); eight cortical layer V (◆) cells and six cortical layer VI (□) cells (lower graph). The probability (Prob.) has been normalized for each cell prior to obtaining the mean responses such that the average probability of each cell was taken as 100% during the period prior to propofol administration.

TABLE II. *Actions of drugs which alter central noradrenergic action. Barb = Duration of barbiturate action, Sleep = length of time animal sleeps after administration, Anaes. = anaesthetic action, estimated from evoked cortical responses when superimposed upon a basal level of urethane. Inc.* for some anaesthetics only (thiopentone, Althesin, ketamine, but not urethane); Inc. = increase; Dec. = decrease; b* = drug does not cross blood-brain barrier; — = not tried. Drug dose is for i.p. administration (for further details see text)*

Drug	Action	Dose (mg kg ⁻¹)	Barb	Sleep	Anaes.
6-Hydroxy dopa	Depletor		Inc.	—	Inc.*
ST587	α ₁ agonist	> 5	Dec.	—	—
Prazosin	α ₁ antagonist	> 0.5	Inc.	—	—
Methoxamine	α ₁ agonist	10	No effect b*	—	—
Clonidine	α ₂ agonist	> 0.125	Inc.	Inc.	Inc.
Guanabenz	α ₂ agonist	> 1	—	—	Inc.
Medetomidine	α ₂ agonist	> 0.01	—	—	Inc.
Phentolamine	α antagonist	40	—	—	No effect b*
Idazoxan	α ₂ antagonist	> 0.1	—	Dec.	Dec.
Yohimbine	α ₂ antagonist	> 0.1	Dec.	Dec.	Dec.
WY 26392	α ₂ antagonist	> 0.5	—	Dec.	Dec.
RS 21361	α ₂ antagonist	> 20	—	Dec.	Dec.
Clenbuterol	β agonist	> 20	Dec.	Dec.	Dec.
Propranolol	β antagonist	> 20	Inc.	Inc.	Inc.
Sotalol	β antagonist	40	No effect b*	—	—
Pindolol	β antagonist	1	Inc.	—	—
Atenolol	β ₁ antagonist	40	No effect	—	—
Metoprolol	β ₁ antagonist	80	No effect	—	—
Salbutamol	β ₂ agonist	20	No effect	No effect	No effect b*
IC 118551	β ₂ antagonist	> 20	Inc.	Inc.	Inc.

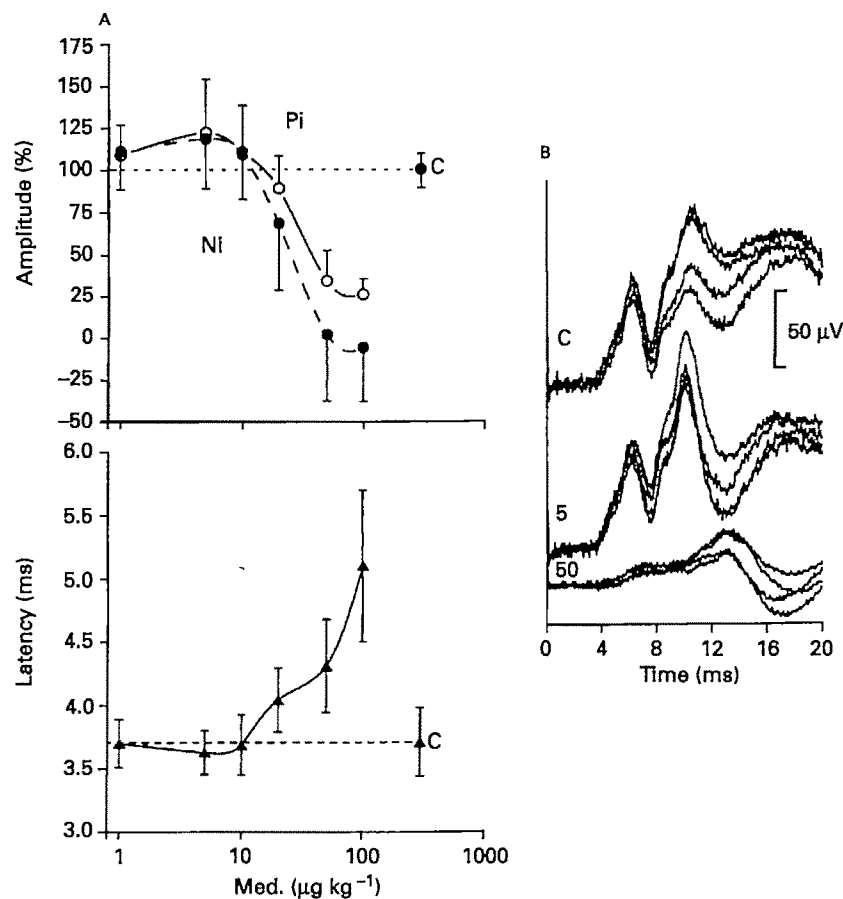


FIG. 12. A: Effect of i.p. medetomidine (Med.) superimposed on a basal concentration of urethane anaesthesia in six rats. C = Control. Data are mean, SD. B: Groups of four averaged cortical evoked responses superimposed (each average of 50 consecutive forepaw stimuli at a rate of 1 s⁻¹) before (C) and after medetomidine 5 µg kg⁻¹ (5) and 50 µg kg⁻¹ (50) i.p.

studied so far, approximately 50% showed an increase in their response probability, 25% showed no change and the remainder showed decreased

responsiveness. This differential behaviour could not be related to their position in the ventrobasal thalamus or to their modality of activation (i.e.

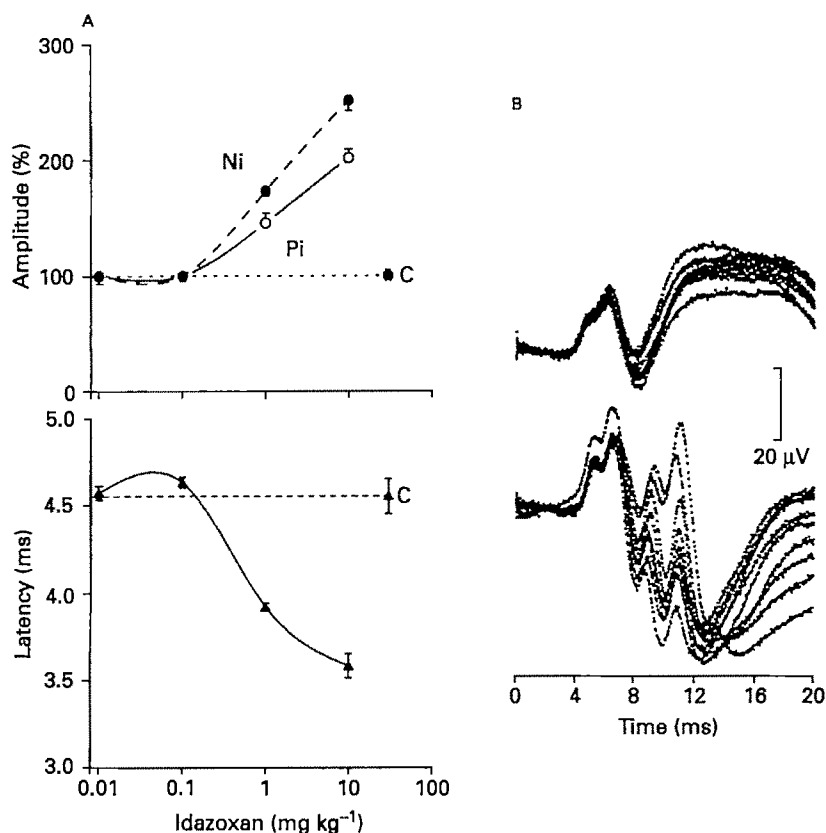


FIG. 13. A: Effect of i.p. idazoxan superimposed on a basal level of urethane anaesthesia in six rats. C = Control. Data are mean, SD. B: Groups of 10 averaged cortical evoked responses superimposed (each average of 50 consecutive forepaw stimuli at a rate of 1 s^{-1}) before (upper responses) and after idazoxan 10 mg kg^{-1} i.p. (lower responses).

touch, gentle pressure or hair movement). Cells in all layers of the cortex showed decreased responsiveness with cortical layer VI cells showing a marked decrease in responsiveness compared to their behaviour after group A anaesthetics.

NORADRENERGIC SYSTEM

One of the first indications of a link between concentrations of central monoaminergic neurotransmitters and anaesthesia came from the work of Miller and colleagues [14, 20], who showed that decreased concentrations were associated with a decrease in halothane MAC and increased concentrations were associated with an increased halothane MAC. Further understanding of the role of central amines in the genesis of the anaesthetic state has had to wait for the development of specific agonists and antagonists to central noradrenergic and 5-hydroxytryptaminergic receptors. The role of the noradrenergic system in sedation and anaesthesia has been the subject of much investigation in my own laboratory and a list of noradrenergic interactions to prolong barbiturate anaesthesia as a definite central effect and to induce a state of sleep or behavioural excitability is shown in table II (see also [7, 17–19]).

In the initial experiments on central noradrenergic mechanisms, it was found that the α_2 adrenoceptor agents had the most clearcut effects. Therefore, an investigation on their action on cerebral evoked responses was carried out. Urethane, apart from having little or no influence on respiratory and

cardiovascular variables in the rat, produces a prolonged state of stable anaesthesia and is metabolized slowly. A stable level of anaesthesia has been measured over periods of 3 h. Thus if a drug is administered which alters the level of anaesthesia in less than this period, it may be said to be exerting a direct effect and not just increasing the metabolic removal of the urethane, especially so if its effect can be antagonized by an appropriate agonist or antagonist (see below). It was found that α_2 adrenoceptor agonists increased the depth of urethane anaesthesia, when assessed by their action on evoked cerebral cortical responses, whereas α_2 adrenoceptor antagonists decreased the level of anaesthesia (see table II). The first demonstration of a possible role for an α_2 adrenoceptor agonist (clonidine) in anaesthesia was shown by Angel and colleagues [4].

Medetomidine, a potent, selective and specific α_2 adrenoceptor agonist has been launched as a powerful sedative and analgesic for veterinary practice and has begun to be used for clinical human anaesthesia. Figure 12 shows the effect of medetomidine when superimposed on a basal level of urethane anaesthesia. At small doses ($< 5 \mu\text{g kg}^{-1}$) it acts to decrease slightly anaesthetic level but at larger doses ($> 20 \mu\text{g kg}^{-1}$) it increases the latency of the evoked cortical response and decreases the amplitudes of the initial positive and negative waves in a dose-dependent fashion (i.e. produces an increased depth of anaesthesia). All α_2 adrenoceptor agonists tested so far (those listed in table

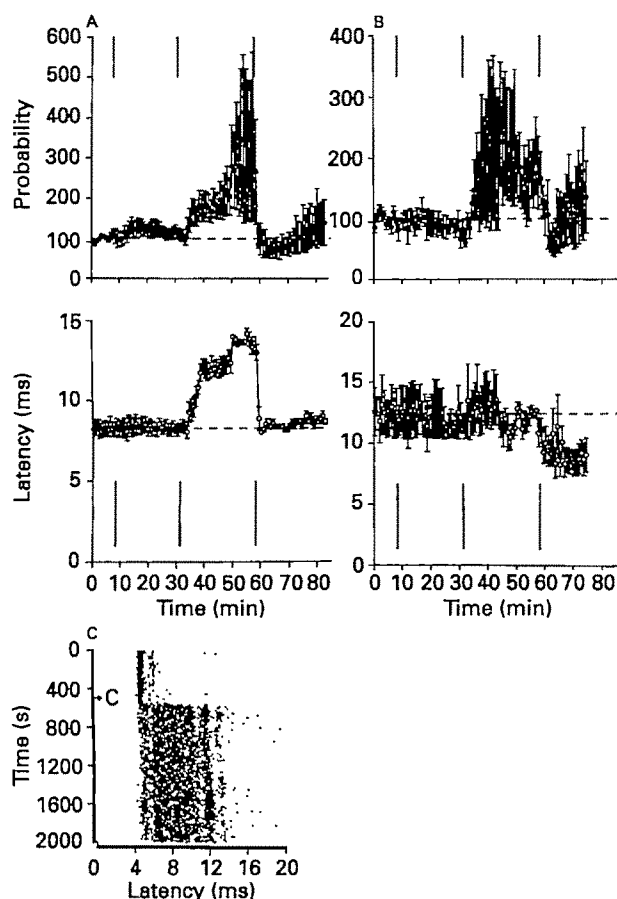


FIG. 14. A: Effect of i.p. injections of medetomidine $5 \mu\text{g kg}^{-1}$ (first vertical line) and $50 \mu\text{g kg}^{-1}$ (second vertical line) and idazoxan 10 mg kg^{-1} (third vertical line) on the probability of response (upper graph) and median latency (lower graph) of seven cortical cells from layer IV. Each cell was normalized to a response of 100% during the period prior to administration and the responses to 50 consecutive stimuli averaged before obtaining the mean responses. Data are mean, SEM. B: The same for four cells from cortical layer V. C: Effect of i.p. injection of clonidine (C) $500 \mu\text{g kg}^{-1}$ on the discharge of a single cell isolated from the ventrobasal thalamic relay nucleus in a rat surgically anaesthetized with urethane. The ordinate shows the response number and since the electrical stimuli were applied to the contralateral forepaw at a rate of 1 s^{-1} , it also shows time. The abscissa shows the latency at which the cell discharged, indicated by a single dot for each action potential generated by the cell. It may be seen that the initial discharge of the cell occurred at a later latency after clonidine but that the cell then continued to discharge. This gives a large increase in probability of response but the thalamocortical volley will be asynchronous.

II) show this biphasic dose-related response on anaesthetic depth. The converse effect is seen with α_2 adrenoceptor antagonists. At small doses they may produce a small increase in anaesthetic depth but at large doses act to decrease the level of anaesthesia; giving a decrease in the latency and increases in the initial positive and negative waves of the evoked cortical response. An example of an α_2 adrenoceptor antagonist (idazoxan) is shown in figure 13. This provides an exciting new prospect for anaesthetic management; of using a powerful drug with known action to produce anaesthesia and its pharmacological antagonist to antagonize anaesthesia. It must be pointed out that the anaesthetic action of these α_2 adrenoceptor agonists is achieved at very large concentrations compared to

the doses used to produce a maximal peripheral effect.

Examination of the two α_2 adrenoceptor agonists clonidine and medetomidine, on the responses of single cells along the somatosensory pathway shows that as with all other anaesthetic agents, they have no effect on the activity of cells at the first sensory synapse, in the dorsal column nuclei. Thalamic cells, however, show a large increase in the probability of responding to a peripheral stimulus, seen as a prolongation of the discharge to a single stimulus with an increase in the latency at which they first respond. The initial discharge is decreased, the initial latency is increased, but the overall probability is increased, thus giving a dispersed discharge such that the thalamo-cortical volley becomes asynchronous (see fig. 14c). The same response change is seen in cells in cortical layer IV (see fig. 14A). Cells in this layer appear to mirror the ascending thalamo-cortical input as they do with group A anaesthetics. This behaviour is also seen with cells in cortical layer V, except that they show no change in latency of response (fig. 14B). These changes in cellular activity could have been predicted from depth-latency responses (see fig. 7B). Figure 14 would also predict that the cellular responsiveness in cortical layers III and VI would show the same effect. Figure 14 also shows that the effect of the α_2 adrenoceptor agonist medetomidine, may be antagonized with an appropriate dose of the α_2 adrenoceptor antagonist idazoxan, indicating that there has been no increase in the metabolism of urethane during the course of the experiments.

The major effect of the α_2 adrenoceptor agonists on sensory transmission appears to be complete disruption of the timing of the signals passed from the ventrobasal thalamus to the cerebral cortex. But once again it is the thalamic transfer which is upset, as with all group A anaesthetics.

5-HYDROXYTRYPTAMINERGIC (5-HT) SYSTEM

Although modulation of the 5-HT system has yet to produce indications for the use of agonist or antagonist drugs as anaesthetics, they are known to modulate sensory transmission. Increases in central 5-HT concentrations with 5-hydroxytryptophan [11] and depletion with para-chlorophenylalanine [8] have been shown to change auditory evoked responses in opposite directions. In the present study, the administration of para-chlorophenylalanine was found to decrease anaesthetic depth and treatment with 5-hydroxytryptophan, which enters central neurones and promotes the release of 5-HT, caused an increase in anaesthetic depth. 5-HT₃ antagonists, which are centrally active, decrease the level of anaesthesia (see table III).

CHOLINERGIC SYSTEM

Both atropine and hyoscine, in large doses, are known to cause drowsiness and central depression. The effect on evoked cortical responses is to cause an increase in anaesthetic depth. That this is a central muscarinic effect is shown by the facts that

TABLE III. *Actions of drugs which alter central 5-hydroxytryptaminergic or cholinergic action. Anaes. = Anaesthetic action, estimated from evoked cortical responses when superimposed upon a basal level of urethane. Inc. = Increase; Dec. = decrease; b* = drug does not cross blood-brain barrier. Drug dose is for i.p. administration (for further details see text)*

Drug	Action	Dose (mg kg ⁻¹)	Anaes.
para-Chlorophenylalanine	5-HT depletor	75	Dec.
5-Hydroxytryptophan	Inc. 5-HT level	75	Inc.
MD 7222	5-HT ₂ antagonist	> 0.001	Dec.
Odanserin	5-HT ₂ antagonist	> 2	Dec.
Atropine	Muscarinic antagonist	> 2	Inc.
Hyoscine	Muscarinic antagonist	> 1	Inc.
Atropine methyl nitrate	Muscarinic antagonist	20	No effect b*
Mecamylamine	Nicotine antagonist	8	No effect

atropine methyl nitrate, which does not cross the blood-brain barrier, is without effect and that a central nicotinic antagonist, mecamylamine, was also without effect.

CONCLUSIONS

Anaesthetics set in train a positive series of events which are targeted at specific sites of synaptic transfer in the sensory pathway. One of their prime actions is to modify the transfer of information across the thalamic sensory relay synapse. This is seen with all anaesthetic agents tested. For group A anaesthetics, this results in a decrease in the amount of information which may gain access to the cerebral cortex. Simplistically, if the sensory message is barred, or impeded on its path to the sensory cortex, the conscious awareness of sensory messages will be diminished. If a fixed amount of information, above some threshold level, is required for conscious translation, then because of their impeding action on sensory transfer, no sensations will be reported, or experienced by patients and thus they will be anaesthetized. For group B anaesthetics, the position is less clear, but a thalamic upset, in addition to inhibition of cortical neurones will give the same end result. For alpha₂ adrenoceptor agonists, the same will be seen because in spite of the increased (overall) response of thalamic sensory relay neurones, the message transferred to the cerebral cortex will be less synchronous, and more dispersed, and this could be interpreted as a garbled message. If diminished or garbled messages reach the cerebral cortex for onward transfer and processing, their action on the sensory pathway could be used to explain the amnesia seen after anaesthetic administration.

The reticular influences on brain stem, basal ganglia, cerebellar and motor cortical neurones would impede the production of volitional movements since the ascending sensory information from kinaesthetic receptors will be disrupted at its thalamic site of transfer and the planning (basal ganglia), programming (cerebellum) and executive motor neurones (motor complex) would be deprived of their appropriate information and its transfer. This effect would be amplified by the large susceptibility of layer V neurones to anaesthetics, since these are involved in the generation of the centripetal message to produce movements. Reticular influences on vestibulospinal, cerebellar and spinal motor neurones could be used to explain disruption in high

level (righting and protective) reflexes, and in stretch reflexes, especially if one theorizes that the action of anaesthetics seen on the reticular control of sensory neurones is the same for motor neurones (i.e. increased inhibition and decreased excitation).

Finally, it should be apparent that mass cortical responses may only provide a limited amount of information about the behaviour of the central nervous system. It is necessary to study events at the single cell level to appreciate the differential effects of anaesthetics. This is exemplified by a comparison of group A anaesthetics and alpha₂ adrenoceptor drugs. The changes in the evoked responses appear similar but their effect on the thalamic relay nuclei are quite different.

APPENDIX

Acute experiments

Anaesthesia. Female rats (Sheffield strain), in the weight range 190–210 g, were anaesthetized with ethyl carbamate (25 % w/v in 0.9 % saline) i.p. without premedication. The anaesthetic depth was adjusted to just abolish reflex withdrawal of the hindlimb to a strong pinch (dose 1.25–1.5 kg⁻¹). At this depth of urethane anaesthesia, arterial pressure and alveolar and arterial oxygen and carbon dioxide tensions are within normal limits [12, 15].

Preparation. After tracheal cannulation, the medulla was exposed by opening the foramen magnum and removing the arch of the first cervical vertebra. The left cortex was then exposed and the dura mater reflected. Any animals that showed signs of cerebral oedema were excluded from the analyses (see [3]). The animals, once prepared, were mounted in a stereotaxic frame and held rigidly by bars inserted into each external auditory meatus to puncture the tympanum and by a bar clamped against the hard palate immediately behind the upper incisors. A leak proof pool was formed by gripping the cut skin edges between an inner perspex ring and an outer circular clip and was filled with liquid paraffin BP which had been saturated with physiological saline at 38 °C. To reduce movements of the exposed nervous tissue to respiratory effort or cardiac pulsation, the animals were suspended, with the body unsupported, between the ear bars and a pin inserted deep to the ligaments covering the dorsal aspect of the pelvic girdle and the trunk was put under slight traction. Rectal temperature was maintained at 37 ± 0.5 °C by circulating water at 40 °C through a hollow copper block placed underneath, but not in contact with, the animal.

Chronic experiments

Under thiopentone-trichlorethylene anaesthesia, and observing full aseptic precautions, three small screws were implanted into the skull: one over the sensory cortical area for the forepaw, the second 4 mm caudal to serve as an indifferent electrode, and the third on the opposite side of the skull to act as an earth electrode. These screws were then wired into a miniature socket and the whole assembly embedded, together with the cut skin edges, in

cold-curing acrylic cement (Simplex Rapid, Dental Fillings Ltd). A period of at least 3 days' recovery was allowed before subsequent experimentation. For recording, the animal was treated with chlordiazepoxide 15 mg kg^{-1} and suspended in a hammock; chlordiazepoxide at this dose level acts as a sedative but does not effect significantly the cortical evoked response.

Techniques

Recordings. Responses from the cerebral cortex, via fine silver wires placed gently in contact with the surface in the acute preparation and from the extradural screws in the chronic preparation, were amplified with resistance-capacity coupled amplifiers and displayed on a cathode ray-oscilloscope or averaged with a microprocessor and stored on floppy discs for subsequent statistical evaluation.

Glass micropipettes of tip diameter $2\text{--}5 \mu\text{m}$ and with tip impedances in the range $2\text{--}8 \text{ Mohm}$ filled with 2% Pontamine Sky Blue in $0.5 \text{ mol litre}^{-1}$ of sodium acetate were used to record extracellularly from single neurones. The activity from the electrodes was led via a short piece of chlorided silver wire into a high input impedance, unity gain FET input amplifier. Spike activity was visualized with conventional recording techniques, and additionally could be monitored via an audio system. Spontaneous and evoked cellular activity was also processed with a microprocessor and data were stored for subsequent statistical manipulations on floppy discs. The microelectrode assembly was mounted on three lathe slides placed mutually at right angles to give positioning in the mediolateral, rostrocaudal and dorsoventral axes. The zero reference point for the stereotaxic co-ordinates in the rostrocaudal and mediolateral planes, set at the beginning of each experiment, was the bregma, the intersection of the sagittal and frontal cranial sutures. The dorsoventral zero was taken as the point at which the micropipette entered the surface of the nervous tissue. When the surface position had been found, subsequent movement of the microelectrode in the dorsoventral axis was controlled by a stepper-motor drive geared such that four steps of the motor advanced the electrode tip $1 \mu\text{m}$. The electrode was usually advanced in 2 or $5 \mu\text{m}$ steps until a cell had been isolated from the background noise and its recorded potential optimized. Iontophoretic ejection of the Pontamine Sky Blue could be used for histological verification of the position of the tip of the recording microelectrode.

Stimulation. The right forepaw was stimulated electrically via gauze strips moistened with $\text{NaCl } 3 \text{ mol litre}^{-1}$, one wrapped around the wrist, the negative electrode, the other around a digit. Care was taken when applying the strips not to occlude the blood supply to the hand. Rectangular pulses of 0.1-ms duration and of variable voltage (from 0 to 90 V), isolated from earth (Devices stimulator type 2533) were delivered at a rate of 1 s^{-1} .

Administration of drugs. Anaesthetic vapours and gases were given in an oxygen stream, saturated with water vapour, and delivered to one arm of a T-piece with the animal's tracheal cannula inserted into the base of the T. The other arm of the T-piece was connected to an anaesthetic venting system.

All water soluble drugs were administered in solution in saline at 37°C via a tail vein, except for ethyl carbamate, which was administered i.p.

Of the gaseous and vaporizable anaesthetics, cyclopropane and nitrous oxide inspired concentrations were determined by measuring the gas flow relative to that of the oxygen flow; diethyl ether, enflurane, halothane, methoxyflurane, isoflurane and trichlorethylene were delivered from calibrated vaporizers (Cyprane Ltd). The other vapours were crudely administered by diverting a measured portion of the oxygen flow through a Dreschel bottle containing the anaesthetic.

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CORRESPONDENCE

BEDSIDE APTT AND PT MONITORING

Sir,—Recently an extensive and interesting review in the *BJA* [1] emphasized the importance of thrombelastography (TEG) for monitoring coagulation in the perioperative period. Whereas TEG investigates haemostasis as a whole dynamic process, activated thromboplastin time (APTT) and prothrombin time (PT) represent isolated end-points in haemostasis and give no information on interaction between platelets and the coagulation cascade [1]. However, we wish to present a clinical report showing that, at least in certain circumstances, bedside "real-time" monitoring of APTT and PT can improve the management of patients by anaesthetists, in the operating theatre.

A 67-yr-old woman, recovering from inferior left limb thrombosis, manifested epigastric pain after eating. Radiological examinations (stomach x-ray and abdomen CT scan) and gastric biopsies demonstrated diffuse infiltrative adenocarcinoma of the stomach extending to the pancreas. A few days later, new thrombosis developed, occluding the deep veins of the inferior right limb. The patient was treated with infusion of heparin (about 25 000 iu day⁻¹), maintaining APTT between 1.5 and 2.5 times normal. The patient was scheduled to undergo surgery and, 2 days before the date appointed for operation, the heparin infusion was reduced to 15 000 iu day⁻¹. Complete thrombosis developed of the right subclavian and internal jugular vein. Heparin infusion was increased and surgery delayed until resolution of the thrombosis. The heparin infusion was reduced (500 iu h⁻¹) only 3 h before operation. At the beginning of surgery, APTT was 18 s (normal values 18–31 s), the International Normalized Ratio (INR) was 1.2 and other variables of haemostasis (platelets, fibrinogen, antithrombin III, fibrin degradation products) evaluated by the central laboratory were within the normal range.

During the operation (gastrectomy and resection of body and tail of the pancreas), serial measurements of APTT and PT were performed on fresh whole blood at the patient's bedside, using a 512 coagulation monitor (Ciba Corning, U.S.A.)—a small, rechargeable, portable instrument. One drop of fresh whole blood is deposited on the surface of a test cartridge containing dry reagents which activate the coagulation process. Results are obtained within 2–3 min and data expressed in seconds, the ratio between the patient's and the normal value and INR. During the operation, the patient received crystalloids 2000 ml and plasma expanders 1000 ml, packed red cells 5 units, 500 ml of blood cells as autotransfusion and fresh frozen plasma 3 units. APTT was 18–140 s, INR in the range 1.25–1.78. Ionized calcium was always within the normal range. Monitoring of APTT and PT allowed rapid assessment of heparin infusion and of dilutional hypo-coagulability, and the subsequent treatment of these conditions. During the operation there was no clinical evidence of haemorrhage. Twelve hours after the end of the operation, heparin infusion was increased to 900 iu h⁻¹ (APTT 1.5 times normal). The postoperative blood loss was considered normal by the surgeons.

Bedside APTT and PT monitoring is reliable and performed easily and quickly. This monitoring aids anaesthetists to manage patients affected by deep venous thrombosis and thrombophilic patients undergoing anticoagulant therapy and who require surgery. APTT was found to correlate significantly with all TEG variables [2] and PT was found to correlate significantly with the number of units of blood transfused [3]. It would be of major interest to investigate if, during haemorrhagic surgical procedures requiring large transfusion, bedside APTT and PT monitoring can help to rationalize the use of blood components.

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Pavia, Italy

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R, Ivankovich AD. Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. *Journal of Clinical Monitoring* 1987; 3: 25–30.

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Sir,—Thank you for the opportunity to reply to this letter.

The management of the acutely bleeding patient is undoubtedly facilitated by the use of "bedside" coagulation tests. It is important, however, that the costs of these tests are balanced against the amount of information gained: for instance, the current U.K. cost of Ciba Corning PT is £3.34 per cartridge and the APTT is £4.20. Whether APTT and PT monitoring alone can rationalize the use of blood products is somewhat debatable, as these tests are limited to the start of clot formation and do not help in assessing its dynamic development, stability and strength. Bedside monitoring of APTT (and PT) would, however, seem to be a major advance for the clinician managing anticoagulated patients during operation or in the intensive care unit.

Before blood product therapy such as fresh frozen plasma is used to correct a prolonged PT and APTT, it is clear that more work needs to be done to define "normal" changes in patients undergoing extensive surgery with and without major bleeding, as striving to keep these variables within tight limits may be both unnecessary and unrealistic. We agree with the authors that it would be of interest to evaluate further the use of bedside testing of PT and APTT in surgical patients.

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SORE THROAT AFTER SUXAMETHONIUM

Sir,—In their paper comparing alfentanil and suxamethonium, used to facilitate intubation for day-case dental surgery [1], Alcock and colleagues express surprise at the greater incidence of postoperative sore throat in those patients receiving suxamethonium [1]. They would have expected this complication to be more common in the alfentanil group, because of the increased manipulation of the tracheal tube sometimes required in these less-well relaxed patients. (Placement of the pharyngeal pack would, similarly, tend to be more traumatic.) The authors are unable to explain this preponderance of sore throat in the suxamethonium group.

I suggest that this discomfort was merely the interpretation by the patient of myalgia affecting the muscles of the pharynx—that is, a local manifestation of the generalized muscle stiffness and pain associated with suxamethonium. It would be instructive to examine the data produced by the study for any possible correlation between sore throat and muscle pain.

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1. Alcock R, Peachey T, Lynch M, McEwan T. Comparison of alfentanil with suxamethonium in facilitating nasotracheal intubation in day-case anaesthesia. *British Journal of Anaesthesia* 1993; 70: 34–37.

RESPIRATORY DEPRESSION AFTER EXTRADURAL FENTANYL

Sir,—The letter by Wang [1] adds one more to at least three existing case reports on ventilatory depression after single extradural injections of fentanyl [2–4]. The attribution of the effect to perispinal vascular communications between extradural and cerebral blood vessels is unconvincing. It is hard to reconcile

with the delay of more than 1 h between administration of fentanyl and hypoventilation [1, 2], and it ignores several other possible causes that others have discussed.

Ventilatory depression may be expected within the first 1 h after extradural injections of larger doses of lipophilic opioids. It may, quite reasonably, be attributed to systemic absorption of most of the dose, although a short latency from injection does not necessarily preclude effects from CSF-borne opioid. Clearly, ventilatory depression can occur more than 1 h after extradural injections of lipophilic opioids, with systemic concentrations considerably smaller than the analgesic range, but latencies longer than 2 or 3 h have not been reported—in contrast with morphine.

In none of the case reports has the dose of fentanyl been suspiciously large. A dose of 200 µg produces a clinically insignificant depression of ventilatory carbon dioxide sensitivity, maximal at 60 min [5]. The same dose i.m. produced no detectable depression, despite producing greater systemic concentrations of fentanyl. Thus the depression seen with the extradural dose was probably caused by fentanyl reaching the brainstem via another route—presumably the CSF—with a contribution from the systemic concentrations which, at 60 min, had reached a maximum within the range for systemic analgesia [6]. Extradural doses of fentanyl, 100 µg or less, and equi-effective doses of other lipophilic opioids, produce concentrations that would not, by themselves, produce analgesia or depression of breathing [7, 8]. If the input of such opioids is continued over a period, and even if one attempts to restrict the dose to the minimum required by the patients, systemic concentrations are liable to increase into the range that produces systemic analgesia and depression of breathing. Thus the occurrence of severe sedation [9] or depression of breathing [10] during the course of continued postoperative infusions of fentanyl is not unexpected.

Rapid injections of large volumes (15–20 ml) of extradural injectate may be expected to promote rostral spread in the extradural space and more rostral penetration into the CSF [11], especially in patients of short stature [12]. This may cause ventilatory depression from CSF-borne opioid that is indistinguishable from the systemic effects, except perhaps for the severity in relation to dose.

Extradural injections in large volumes may account for the cases of early or intermediate-latency ventilatory depression reported with methadone 4 mg [13], buprenorphine [14], sufentanil 50 µg [15] or hydromorphone 1.25 mg [16]. Slower rates of injection or divided injections of much larger doses have been tolerated much better (for example an accidental infusion of sufentanil 180 µg [17]).

In none of the four case reports of ventilatory depression after extradural fentanyl was the dose (90–100 µg) given rapidly in a large volume, although effects were detected as high as T2 [1] and C4–5 in two patients [2]. In one patient [4], the disturbance caused by lithotripsy was invoked as possibly accelerating cephalad spread of the modest volume of extradural injectate. That patient also received droperidol (and midazolam and nalbuphine) systemically, and the administration of the antiemetic metoclopramide [1, 2] was a suspiciously common feature in two of the remaining three (the third received diamorphine in addition to the extradural fentanyl [3]). Droperidol was also implicated in the report by Cohen, Rothblatt and Albright of ventilatory depression 15 min after extradural hydromorphone [16].

It is tempting to postulate some interaction of antiemetics with depressant effects of opioids on breathing (possibly through modulation of central catecholamine release by actions on dopaminergic receptors [18]). There is no particular suspicion of any such interaction with systemically administered opioids, so the postulate would have to include the presence of relatively large concentrations of opioid in the CSF bathing the brainstem. Wells and Davies [4] reported hypoalgesia extending to the face as evidence of large concentrations of opioid bathing the nuclei of the cranial nerves. Sudden occurrence of severe nausea and onset of vomiting may be corroborating evidence. If opioid-induced vomiting were the prelude to both ventilatory depression and administration of an antiemetic, the association between the depression and the antiemetic may need no further explanation. However, it is notable in the case reported by Cohen, Rothblatt and Albright [16], that the vomiting and the administration of droperidol preceded administration of extradural hydromorphone.

Ideally, the postulate of a ventilatory depressant interaction between systemic antiemetic and CSF opioid ought to be tested by

a randomized controlled trial. However, any such trial would involve almost impossibly large numbers because administration of antiemetics with extradural opioids is relatively common and the incidence of late onset ventilatory depression is relatively rare. Better information on the possible risk may be obtained by a request to all anaesthetists to report all cases of ventilatory depression with extradural fentanyl, with or without concurrent administration of antiemetics. Alternatively, the centres that use extradural opioids should co-operate in a multicentre prospective audit to examine questions of this type.

Meanwhile, the utmost vigilance should be maintained when using extradural opioids, but particularly when the use is accompanied by sedation, nausea, vomiting or the use of antiemetics.

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1. Wang CY. Respiratory depression after extradural fentanyl. *British Journal of Anaesthesia* 1992; 69: 544.
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Sir,—The suggestion of a ventilatory depressant interaction between systemic antiemetic (in this case metoclopramide) and CSF opioid is interesting.

Metoclopramide has been used extensively for the treatment and prevention of nausea and vomiting, in the past 30 years and there is an extensive literature on its efficacy. Recognized side effects of metoclopramide include extrapyramidal reactions, sedation, restlessness and cardiovascular effects. However, respiratory depression has not been reported and high-dose metoclopramide has been used in the prevention of nausea and vomiting in patients receiving chemotherapeutic agents [1].

Chrubasik, Chrubasik and Black have concentrated their comments on the dosage and the time course of respiratory depression. The respiratory depression observed after extradural fentanyl may be caused by three mechanisms alone or in combination: a systemic effect; rostral spread in the CSF [2]; rostral spread via a direct perimedullary channel [3]. A systemic effect seems unlikely. It is generally accepted that fentanyl, a highly lipid-soluble opioid, easily crosses the dura and quickly penetrates the spinal cord, inducing segmental analgesia without the side effects associated with migration of the opioid in a rostral direction [3]. However, the study by Gourlay and colleagues [2] demonstrated rapid (30 min) appearance of large concentrations of fentanyl in cervical CSF after lumbar extradural, but not in i.v., fentanyl injection. The mechanism for this rapid redistribution is uncertain, but it may explain cases of rapid-onset, severe respiratory depression from lipid-soluble opioids. In my case report, respiratory depression occurred 80 min after the initial injection of bupivacaine and fentanyl.

It seems probable, therefore, that the respiratory depression in this patient was caused by the larger doses of fentanyl being administered, enhancing rostral spread via a direct perimedullary vascular channel [3].

I agree strongly with Drs Chrubasik, Chrubasik and Black that the utmost vigilance should be maintained with every use of extradural opioids.

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NEUROMUSCULAR BLOCK AND INSERTION OF THE LARYNGEAL MASK AIRWAY

Sir,—We have been following the recent correspondence about hypoxaemia and laryngeal mask airway (LMA) insertion and were interested in the comments on insertion under neuromuscular block [1, 2]. Hayes, Allsop and Gillies found that insertion of the LMA was difficult in 16 of 87 patients after induction of anaesthesia with propofol 2 mg kg⁻¹ and fentanyl 1 µg kg⁻¹ [3]. In contrast, Yaddanapudi and colleagues found that thiopentone 3-6 mg kg⁻¹ and suxamethonium 1.5 mg kg⁻¹ resulted in only one difficult insertion in 20 subjects [1]. Based on these results, Yaddanapudi's group suggested that insertion of the LMA under neuromuscular block was a viable alternative to insertion with

propofol and might be considered when the requirements of surgery necessitated controlled ventilation.

As Gillies commented [2], it is difficult to make direct comparisons between separate studies with different designs. We present data from the control groups of the two LMA insertion studies we have conducted and which followed similar designs and incorporated standardized criteria for successful insertion. From these data, we feel that a more conclusive statement can be made regarding ease of insertion of the LMA with propofol and fentanyl compared with thiopentone and suxamethonium.

Ethics Committee approval and informed consent were obtained for both studies. Control group A comprised 40 patients in whom anaesthesia was induced with fentanyl 1 µg kg⁻¹ followed, 2 min later, by propofol 2.5 mg kg⁻¹. In control group B, 50 patients underwent induction of anaesthesia with thiopentone 4-6 mg kg⁻¹ and, after loss of consciousness and lash reflex, were given suxamethonium 1 mg kg⁻¹. All the patients were fasted, ASA I-II and were monitored with an ECG, pulse oximeter and Dinamap. All patients were preoxygenated for 3 min, with no subsequent manual ventilation.

A standard insertion technique was used in both groups [4]. In control group A, LMA insertion was attempted 1 min after completion of propofol injection. In control group B, LMA insertion was attempted when fasciculations had ceased. When the LMA was in position, a fiberoptic scope was passed to the level of the mask aperture bars and the view scored [5]. Function was assessed by gentle hand ventilation and synchronous observation of chest wall movement.

The mean age, weight and male:female ratio were similar in the two groups. The results for fiberoptic scoring are shown in table 1. In all patients, oxygen saturation remained greater than 94%. In control group A, all LMA were inserted within 10 s of placing the bowl of the LMA in the mouth to inflation of the cuff. In control group B, there was one failure and in all other patients the insertion time was between 5 s and 18 s from the time the LMA was picked up to inflation of the cuff. The distribution by fiberoptic scoring between control group A and group B was analysed using the chi-square test and revealed no significant difference.

TABLE 1. Results for fiberoptic scoring. Score 4 = only cords; 3 = cords plus posterior epiglottis; 2 = cords plus anterior epiglottis; 1 = cords not seen, but function adequate; 0 = failure to insert within 60 s or to function

	Score					Median score
	4	3	2	1	0	
Group A	23	10	6	1	0	3.4
Group B	26	6	10	7	1	3.0
Total	49	16	16	8	1	3.2

These data suggest that there is no difference between ease of insertion of the LMA using propofol 2.5 mg kg⁻¹ and fentanyl 1 µg kg⁻¹ compared with thiopentone 4-6 mg kg⁻¹ and suxamethonium 1 mg kg⁻¹. It is possible that smaller doses of propofol, as used by Haynes, Allsop and Gillies [3] may make conditions less favourable for LMA insertion, although Blake and colleagues found no difference in ease of insertion of the LMA when comparing a dose of 2 mg with 2.5 mg at 90 s [6].

It would seem that neuromuscular block provides good conditions for LMA insertion, but no better than those provided by an adequate dose of induction agent. Given the lack of improvement in insertion conditions, there would appear to be no advantage in routinely using neuromuscular blocking drugs to aid insertion. We also suggest that, if an adequate dose of induction agent has been administered and there is subsequent difficulty in LMA insertion, suxamethonium would not improve conditions for insertion. If tracheal intubation has failed, however, it is useful to know that LMA insertion can be achieved easily if the patient is still paralysed.

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2. Gillies GWA. Hypoxaemia and insertion of the laryngeal mask airway. *British Journal of Anaesthesia* 1992; 69: 661.
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Sir,—Thank you for the opportunity to respond to the letter of Drs Brimacombe and Berry. Their results clearly indicate that an adequate dose of induction agent provides as good insertion conditions for the laryngeal mask airway (LMA) as neuromuscular block. We have not suggested that neuromuscular block be used routinely in all patients. We feel that an adequate depth of anaesthesia is necessary before an attempt is made to introduce the LMA. Where the surgical requirements demand muscular relaxation and controlled ventilation, we see no harm in inducing neuromuscular block before securing the airway with an LMA as an alternative to either an adequate dose of i.v. anaesthetic or an inhalation agent.

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PREOPERATIVE FASTING GUIDELINES FOR CATARACT SURGERY UNDER REGIONAL ANAESTHESIA

Sir,—We welcome the editorial by Professor Strunin [1] on preoperative fasting guidelines, recommending clear fluids in healthy patients undergoing elective surgery. We have gone further for patients who undergo cataract surgery; they are often elderly and suffer from systemic disease which requires medication. At the Gimbel Eye Centre in Calgary, all cataract surgery is conducted under regional anaesthesia, either retrobulbar or peribulbar block, and sedation is required in less than 1% of the patients. Since 1984, all patients have been advised to have their normal breakfast, including solids if this is their habit, and medications on the morning of surgery. There is therefore no change in their daily routine. This is particularly appreciated and satisfactory for diabetic patients who account for approximately 12% of all our cataract patients. "Pure" regional blocks (without sedation) are also used at Foothills Hospital, where the same preoperative regimen was adopted in 1988. Our combined experience now extends to more than 30 000 patients, with no case of aspiration of gastric contents. This number includes 14 patients, eight of whom lost consciousness, in whom central spread of the local anaesthetic occurred [2]. No measurements of gastric fluid volume or pH have been made and the absence of complications does not mean that they cannot occur. Nevertheless, the statistical "rule of three" (3/n) [3] means that the long-term incidence will not be greater than 3:30 000 or 1:10 000.

We emphasize that these patients do not have heavy sedation, and the regional block is not converted to a general anaesthetic. For this particular group of patients, we therefore recommend no preoperative fast and encourage full diet. In contrast, we do not allow solid food for patients undergoing major regional blocks such as brachial plexus or extradural block which may require i.v. sedation or conversion to general anaesthesia. For those patients, we endorse Professor Strunin's recommendation of clear fluids only on the day of surgery.

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FASTING BEFORE ANAESTHESIA

Sir,—While interested in challenging unfounded dogma [1], we should not discard accepted practice until better methods are demonstrated. In addition, new ideas can easily be misconstrued.

According to our local newspaper [2], not only can you "win an op on the radio" through last-minute cancellations by other patients for operations on "such ailments as varicose veins, hernias and ingrowing toenails", but Sidcup hospital advised that "as these patients will receive only a light anaesthetic, it shouldn't matter if they have already eaten"!!! This message was spread to more than 75 000 people—the newspaper's claimed distribution.

The two patients who died in Mendelson's report [3] had ingested "full meals" 6 and 8 h before anaesthesia: they died because of suffocation on the delivery table. I hope that history unlearned is not relearned.

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ANAESTHESIA FOR NASOTRACHEAL INTUBATION IN DENTISTRY

Sir,—Two papers in the same issue report success rates of 90% [1] and 83% [2] for nasotracheal intubation in dental practice using propofol and alfentanil. Alcock and colleagues [1] wished to avoid possible excessive cardiovascular depression with a larger dose of propofol and Coghlan, McDonald and Csepregi [2] did not wish to use topical local anaesthesia.

I anaesthetize day-case patients using tracheal intubation in an isolated dental unit and wish to avoid myalgia and adverse reactions associated with the use of suxamethonium.

The publication by Guidon-Attali and colleagues [3] reporting 100% success using propofol 3.5 mg kg⁻¹ and topical anaesthesia led me to change my practice. Adult patients requiring intubation are placed supine to prevent haemodynamic consequences, continuous monitoring of ECG, non-invasive arterial pressure and pulse oximetry are commenced and patients receive alfentanil 500 µg. Those scheduled to undergo multiple extractions are given an oral NSAID before anaesthesia.

Propofol 3.5 mg kg⁻¹ is administered and at laryngoscopy the cords are sprayed with 10% lignocaine; a further bolus of up to 50 mg may be required to ensure successful intubation. Anaesthesia is maintained by spontaneous breathing of a mixture of nitrous oxide, oxygen and enflurane.

Using this technique, successful intubation without either significant myalgia or response to intubation is the rule and recovery is not delayed.

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1. Alcock R, Peachey T, Lynch M, McBwan T. Comparison of alfentanil with suxamethonium in facilitating nasotracheal intubation in day-case anaesthesia. *British Journal of Anaesthesia* 1993; 70: 34-37.
2. Coghlan SFE, McDonald PF, Csepregi G. Use of alfentanil with propofol for nasotracheal intubation without neuro-

muscular block. *British Journal of Anaesthesia* 1993; 70: 89–91.

- Guidon-Artali C, Mouillac F, Quilichini D, Paut O, François G. Propofol as the main anaesthetic agent in dental surgery. *Acta Anaesthesiologica Scandinavica* 1990; 34: 397–399.

Sir,—Thank you for the opportunity of replying to the comments of Dr Lake. We agree that the use of larger doses of propofol and the additional use of 10% lignocaine may improve the success rate for intubation. However, it is still our view that the use of topical anaesthesia is inappropriate when intraoral surgery is performed, especially when procedures may be very short in duration.

We were also interested to read the paper by Alcock and colleagues [1] who compared the use of suxamethonium with alfentanil for nasotracheal intubation. They had a similar success rate of intubation (90%) as ourselves (83%) when using a combination of alfentanil and propofol. Interestingly, they suggested that the technique was unsuitable if difficulty in intubation was anticipated. However, we believe that the technique, when used by experienced operators, may be useful in the management of an anticipated difficult airway or intubation, for two reasons. First, assessing the ability to maintain an airway with a facemask is facilitated. Second, if jaw relaxation is not a problem, a "quick look" laryngoscopy to assess ease of intubation or otherwise is usually straightforward. The advantage of this technique is that, should problems occur, the effects of alfentanil may be antagonized rapidly by naloxone.

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- Alcock R, Peachey T, Lynch M, McEwan T. Comparison of alfentanil with suxamethonium in facilitating nasotracheal intubation in day-case anaesthesia. *British Journal of Anaesthesia* 1993; 70: 34–37.

THROMBELASTOGRAPHY IN THE MANAGEMENT OF CARDIOPULMONARY BYPASS FOR A PATIENT WITH REDUCED PROTHROMBIN CONVERSION BECAUSE OF A RARE FAMILIAL HAEMOSTATIC DEFECT

Sir,—We read with interest the recent review on the subject of thrombelastography [1], which provided global and dynamic assessment of haemostatic function from a single blood sample. We describe a case in which the thrombelastogram (TEG) proved helpful in a patient presenting for coronary artery bypass graft surgery who had an unusual inherited haemorrhagic disorder.

A 50-yr-old woman required emergency cardiac surgery for unstable angina after an inferior myocardial infarct. Thrombolytic therapy had not been given because she had been diagnosed previously as suffering from a rare familial haemorrhagic disorder. The condition, described originally by Parry, Giddings and Bloom in 1980 [2], is characterized by reduced prothrombin conversion as shown by grossly prolonged prothrombin consumption index. All known plasma coagulation factors are present in adequate concentrations. *In vivo* and *in vitro* studies suggest the abnormality results from the presence of an inhibitor of the interaction between plasma and phospholipid during blood coagulation [2]. Fresh frozen plasma, platelets and blood transfusion have been necessary in some patients after surgery [2]. The diagnosis in our patient had been made after investigation of her son who, aged 7 yr, had required blood transfusion for excessive bleeding after tonsillectomy. She herself had bled for 24 h after dental extraction, but had undergone cholecystectomy without excessive bleeding.

As there are no previous reports of anaesthesia for cardiopulmonary bypass (CPB) in patients with this condition, advice about anticoagulation was obtained from the consultant haematologist, who had liaised with the referring hospital. Before

operation, conventional tests of coagulation were normal, as would be expected in this condition. The antifibrinolytic agent tranexamic acid 1 g four times a day orally was prescribed. Anaesthetic management was according to normal practice in our institution. Preoperative TEG and activated clotting time (ACT) before systemic heparinization were within normal limits. ACT was 460 s after administration of heparin 3 mg kg⁻¹ before the onset of CPB. An additional heparin 10 000 units was administered during CPB to maintain the ACT greater than 480 s. After the patient was weaned from CPB, protamine sulphate 250 mg was given, with restoration of ACT to 132 s. TEG performed at the time showed evidence of excessive fibrinolysis which reverted to normal after administration of an additional dose of tranexamic acid 500 mg i.v. A cell-saver was used to reinfuse shed and pump blood. There was no evidence of excessive bleeding in the post-bypass period (360 ml over 24 h), and no blood products were administered.

CPB induces several complex disturbances in the coagulation and fibrinolytic system which are not detected by routine coagulation tests; the TEG has been shown to be more useful in this situation [3]. It appears that systemic heparinization and CPB are safe in this very rare condition. The use of antifibrinolytic therapy prophylactically and guided by the TEG in the post-bypass period may be beneficial. We were able to avoid the empirical use of fresh frozen plasma and platelets.

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- Spies BD, Tuman KJ, McCarthy RJ, DeLaria GA, Schillo R, Ivankovitch AD. Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. *Journal of Clinical Monitoring* 1987; 3: 25–30.

LARYNGEAL SPASM

Sir,—The recent article [1] and Editorial [2] in *British Journal of Anaesthesia* prompt me to write concerning the treatment of post-anaesthetic laryngospasm. The treatment which is usually suggested is to administer oxygen under pressure using a face mask, and if dangerous desaturation is occurring, to re-intubate the trachea after injection of an induction agent and suxamethonium. Applying oxygen under pressure to an edentulous, elderly patient lying on his side may be difficult and does not relieve the patient's discomfort and even terror; further, reintubation may be followed by recurrence of the laryngospasm when the tube is removed again.

For many years, I treated post-anaesthetic laryngospasm effectively by injecting a small dose of pethidine i.v. My rationale was that I was probably reducing the sensitivity of the afferent limb of the reflex arc which caused the spasm. However, since the advent of Diazemuls, I have used this drug i.v. to treat cases of laryngospasm which do not respond rapidly to more simple methods such as removing secretions from the pharynx and turning the patient to a lateral position. The benzodiazepine produces an almost immediate cure. In addition to relaxing the larynx, it also calms the patient and reduces the greatly increased oxygen demand caused by frantic efforts to breathe. I no longer attempt to aspirate secretions from the posterior pharynx in such patients, as this may exacerbate the spasm; instead, I wait until the larynx is relaxed and then persuade the patient to inhale quietly then to cough and clear the secretions, which can be expectorated or aspirated from the mouth.

Of course, this treatment of laryngospasm by depressing upper airway reflex sensitivity carries the theoretical risk of encouraging inhalation of regurgitated gastric contents or other material. However, as stated in the Editorial [2], "although aspiration after anaesthesia may be a relatively frequent event after anaesthesia, serious complications appear to be rare". In contrast, serious hypoxaemia caused by laryngospasm may be a very serious event in a patient with, say, compromised coronary circulation.

The use of i.v. diazepam to treat laryngospasm ought to be better known and more frequently used.

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1. Murphy PJ, Langton JA, Barker P, Smith G. Effect of oral diazepam on the sensitivity of upper airway reflexes. *British Journal of Anaesthesia* 1993; 70: 131–134.
2. Drummond GB. Upper airway reflexes. *British Journal of Anaesthesia* 1993; 70: 121–123.

Sir,—Thank you for the opportunity of replying to Dr Gilbertson's letter.

Life-threatening laryngospasm produces strong closure of the glottis which is maintained well beyond the cessation of mucosal irritation [1]. Laryngospasm consists, neurophysiologically, of prolonged tonic adductor spike and muscle activity [1]. The sensitivity of laryngeal adductor neurones involved in the production of laryngospasm has been investigated by Ikari and Sasaki [2]. They found that the threshold of the laryngeal adductor neurones varies sinusoidally during spontaneous ventilation, with laryngeal closure occurring more readily during the expiratory phase. They also found that increasing P_{aCO_2} decreases the reactivity of adductor neurones, and laryngospasm is more likely to occur in hypocapnoeic states. Interestingly, severe hypoxaemia (P_{aO_2} less than 6.6 kPa) was found to diminish the excitability of adductor neurones, such that laryngospasm was inhibited.

In further work that we have conducted on the sensitivity of upper airway reflexes, we have investigated sensitivity after Diazemuls 0.2 mg kg⁻¹ i.v. in healthy, normal volunteers. We found a highly significant depression of the sensitivity of airway reflexes occurring maximally at 5 min after injection and lasting approximately 30 min.

I believe that many of the agents that we use in anaesthesia may have quite different actions on the complex brainstem reflexes controlling upper airway reflexes, and although I do not have any personal experience in the use of i.v. benzodiazepines to treat life-threatening laryngospasm as described by Dr Gilbertson, this does concur with our experimental findings in volunteers.

J. A. LANGTON
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Leicester

1. Suzuki M, Sasaki CT. Laryngeal spasm: a neurophysiologic redefinition. *Annals of Otolaryngology* 1977; 86: 150–157.
2. Ikari T, Sasaki CT. Glottic closure reflex: control mechanisms. *Annals of Otolaryngology* 1980; 89: 220–224.

FACE MASKS AND ANAESTHESIA

Sir,—We are concerned that Dr Wildsmith does not understand the motives for presenting our work on the use of face masks during regional anaesthesia [1, 2]. We must respond by asserting that the objective of our work was to help achieve greater understanding of some of the factors involved in the safe administration of regional anaesthesia. Specifically, our study was designed to examine the role of speech in the dispersal of upper airway bacteria. In common with many who present their work, it is our hope that subsequent practice, even if unaltered, may at least be performed on a more rational basis. We do not wish to discourage the use of face masks as a consequence of our work, but simply to encourage the examination of the effect of speech on bacterial dispersal. We feel that if the taking of a simple precaution is indeed prudent, as Dr Wildsmith suggests, then this practice will stand up to a little scientific scrutiny.

S. W. O'KELLY
D. MARSH
Queen Alexandra Hospital
Portsmouth

1. O'Kelly SW, Marsh D. Face masks and spinal anaesthesia. *British Journal of Anaesthesia* 1993; 70: 239.

2. Wildsmith JAW. Face masks and spinal anaesthesia. *British Journal of Anaesthesia* 1993; 70: 239.

Sir,—I regret that Drs O'Kelly and Marsh have misunderstood me. I did not question directly their motives for presenting their work regarding the use of face masks during regional anaesthesia. What I was questioning were the motives that drive many of our colleagues to find an excuse for not wearing a face mask during such procedures. It is a simple, harmless precaution that has been shown to reduce bacterial contamination of a surface close to the Anaesthetist's face.

J. A. W. WILDSMITH
Royal Infirmary
Edinburgh

ARTERIAL PRESSURE MEASUREMENT IN PREGNANCY

Sir,—Hasan, Thomas and Prys-Roberts [1] concluded that, in patients on the labour ward "Dinamap diastolic arterial pressure must be corrected using a regression equation, or simply by adding 10 mm Hg, before being compared with the available normal and hypertensive arterial pressure values". This may be a valid conclusion to this limited study, but is inappropriate in primigravid patients with more severe pre-eclampsia. The problems of the use of automated, oscillometric devices in patients with severe pre-eclampsia have been raised previously [2], although there is considerable concern within the obstetric community following the recording of more than 500 eclamptic episodes by the British Eclampsia Survey Team in a national audit in 1992 [to be published].

In this study the group consisted of 28 patients; five had hypertension at some stage of the pregnancy and four of those had proteinuria, although this was not quantified. Seven patients had extradural anaesthesia and in the series of observations it appeared that only two patients had arterial pressures greater than 150 mm Hg, and only one patient had a pressure greater than 95 mm Hg. It is not evident if any patient had pre-existing hypertension with superimposed pre-eclampsia. Whether the patients were nulliparous or parous was not stated. Each of these observations has a bearing on the compliance of the circulation at the time of study. The marked variation between the techniques may reflect concurrent uterine activity, variation between midwives and the non-contemporary observations between the automated device and the midwife.

In a study [3] of antepartum, primigravid patients with severe proteinuric hypertension (> 3 g of proteinuria per 24 h) ($n = 17$) and normotensive primigravid patients ($n = 40$) at 32–42 weeks gestation, automated oscillometric observations were compared with *contemporaneous* auscultatory observations. The mean differences between phase 4 of the vascular sounds and the parameter identification point for diastolic arterial pressure was 14.8 mm (+2.9) in the hypertensive group and 7.5 mm (+1.9) in the normotensive group ($P < 0.001$). The mean differences between phase 1 and the parameter identification point for systolic arterial pressure were 5.4 mm (+1.4) and 2.4 mm (+0.9), respectively ($P < 0.01$). "Systolic" and "diastolic" differences of 20 mm Hg and 35 mm Hg, respectively were recorded in two patients with proteinuria of 6 g/24 h.

As the authors indicate, the guidelines for the management of hypertensive diseases in pregnancy are based on studies using phase 4 of the vascular sounds. Taken together with the widespread use of automated oscillometric device in labour wards in this country, particularly the Critikon 845/1846, there may be some cause for concern. Many automated devices display cuff pressure in 8–10 mm decrements to minimize the duration of the inflation–deflation sequence and local morbidity. Some recent models have the capability of displaying cuff pressure at 1–2 mm decrements during cuff deflation. Such devices permit contemporaneous oscillometric and auscultatory observations, allowing the midwife the opportunity of regular automated observations and the ability to compare them with auscultatory observations without changing cuffs. With the large number of eclamptic episodes occurring in England and Wales, the prevalence and aetiology of which are yet to be explained, this would seem to be the minimum precaution that should be adopted to avoid being

"misled" by the automated oscillometric device to which many clinical staff have considerable loyalty.

M. QUINN
Ysbyty Singleton
Swansea

1. Hasan MA, Thomas TA, Prys-Roberts C. Comparison of automatic oscillometric arterial pressure measurement with conventional auscultatory measurement in the labour ward. *British Journal of Anaesthesia* 1993; 70: 141-144.
2. Quinn MJ. Blood pressure measurement in pregnancy. *Lancet* 1991; 338: 130.
3. Quinn MJ. Comparison of automated and auscultatory observations of blood pressure in severe preeclampsia. *Hypertension in Pregnancy* 1993; (in press).

Sir,—We note from Dr Quinn's letter that he concurs with our general finding that measurements from automatic non-invasive arterial pressure recorders differ consistently from auscultatory measurements, whether contemporaneous or not, made by midwives. The differences between his results and ours are small. We did not recruit specifically hypertensive patients, as we wished to study patients with a range of arterial pressures. The statistical methods which we used (Bland-Altman) are the most appropriate to identify factors which vary with the absolute arterial pressure. We detected no such variation. Unfortunately, Dr Quinn's letter and references give us no opportunity to compare his methods or statistical analyses with ours. It is quite possible that differences between his values and ours are caused by dissimilar techniques.

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THORACIC ELECTRICAL BIOIMPEDANCE OR THERMODILUTION FOR MEASUREMENT OF CARDIAC INDEX

Sir,—Young and McQuillan compared cardiac index (CI) in critically ill patients with severe sepsis using thoracic electrical bioimpedance (TEB) and the so called "golden standard", thermodilution (TD). [1] To compare these two methods to obtain CI is akin to comparing apples and peaches. We also have tested TEB against TD during IPPV [2], apnoea, large tidal volume, slow frequency, small tidal volume with high ventilatory frequencies [3], during PEEP-ventilation [4], in spontaneous breathing patients after operation [5] and in patients with sepsis [6]. At that time it was not usual to use the method of Bland and Altman [7] and we used correlation coefficients and regression analysis. In septic patients ($n = 5$), the correlation coefficient was $r = 0.70$; in patients with sepsis undergoing ventilation with a PEEP of 8 cm H₂O, the correlation was worse: $r = 0.57$.

There are important limitations to comparing TEB with the dilution methods such as Fick, dye or thermodilution, which have similar theoretical and practical problems. For example, the Stuart-Hamilton equation used for calculation of CI is correct only in continuous flow conditions [8], but flow is rarely continuous on the right side of the heart where TD measures impedance changes over a few seconds and calculates blood flow over 1 min. Possible faults in calculating CI may derive from: injectate volume, injectate temperature, injection time, temperature loss in the catheter wall or to the vessels during low-flow conditions, temperature changes during inspiration and expiration during artificial ventilation, particularly with large tidal volumes and increased body temperatures in septic patients, and recirculation [9]. TEB measures on the left side of the heart and values are averaged over 16 heart beats or over 1 min. During controlled ventilation there are sinusoidal variations in CI related to intrapulmonary pressure and venous return, which are more pronounced on the right side of the heart [10].

Young and McQuillan took expiration for TD measurements that use one point in this cyclical fluctuation. This improves reproducibility, but decreases absolute precision. For TEB, they

used averaged values over 1 min. With such a design, it is not possible to compare CI values with both methods. Most importantly, it is not possible to assess the accuracy of the TD method when CI is great, because there is no absolute method of measuring cardiac output. For reasons noted above, the accuracy of the TD method must decrease when CI is increased. We found also that in septic states the mean thoracic impedance was small because of intrapulmonary shunting and non-cardiogenic oedema. Z_0 in the denominator of the equation of Sramek for calculation of CI influences absolute values of CI less because the dx-signal, with its first derivative in the numerator, changes in the same direction in the different thoracic fluid content states.

We have used TEB for several years for monitoring septic patients, with the result that we no longer use pulmonary artery catheters. It is the trend of change with medical treatment that is important and the TEB follows such changes accurately.

Cardiac index is only one of the haemodynamic variables obtainable. It is also possible to assess indices of contractility which are increased before there is clinical evidence of sepsis. Furthermore, it is possible to calculate peripheral resistance and, with the latest software, oxygen delivery and consumption.

G. CASTOR
J. SIMON
Caritas Hospital
Dillingen, Germany

1. Young JD, McQuillan P. Comparison of thoracic electrical bioimpedance and thermodilution for the measurement of cardiac index in patients with severe sepsis. *British Journal of Anaesthesia* 1993; 70: 58-62.
2. Castor G, Motsch J, Altmayer P, Helms J, Molter G, Niedermark I. Clinical evaluation of cardiac output measurement by thoracic electrical bioimpedance. *European Journal of Pharmacology* 1989; 36: 38.
3. Castor G, Helms J, Niedermark I, Molter G, Motsch J, Altmayer P. Influence of the ventilatory cycle on cardiac output determination during mechanical ventilation. A comparison of noninvasive electrical bioimpedance with standard thermodilution method. *American Journal of Noninvasive Cardiology* 1991; 5: 57-61.
4. Castor G, Altmayer P, Helms J, Niedermark I, Molter G. Determination of cardiac output during positive endexpiratory pressure. Noninvasive electrical bioimpedance compared to standard thermodilution. *Critical Care Medicine* 1990; 18: 544-546.
5. Castor G, Stoll M, Helms J, Niedermark I, Molter G. Simultaneous cardiac output by thermodilution versus electrical bioimpedance and Doppler ultrasound. *Intensive Care Medicine* 1990; 16: 1.
6. Castor G, Altmayer P, Molter G, Müller D, Simon J. Measurement of cardiac output with the aid of non-invasive electrical bioimpedance in comparison with the thermodilution method. *Anästhesie Intensivtherapie, Notfallmedizin* 1988; 12: 339-344.
7. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1987; 1: 307-310.
8. Cropp GJA, Burton AC. Theoretical considerations and model experiments on the validity of indicator dilution methods for measurements of variable flow. *Circulation Research* 1965; 18: 26-48.
9. Wessel HU, Paul MH, James GW, Grahn AR. Limitations of thermal dilution curves for cardiac output determinations. *Journal of Applied Physiology* 1971; 5: 643-652.
10. Armengol J, Man GCW, Balsys AJ, Wells AL. Effects of the respiratory cycle on cardiac output measurements. Reproducibility of data enhanced by timing the thermodilution injections in dogs. *Critical Care Medicine* 1981; 9: 852-854.

Sir,—Drs Castor and Simon correctly outline the technical problems involved in the measurement of cardiac index using thermodilution, but miss the point of our paper.

We compared transthoracic impedance and thermodilution methods to measure cardiac index. We make no claims that thermodilution is a "gold standard" and, in fact, the statistical method of Bland and Altman that we used is specifically designed

for the comparison of two methods of measurement when neither is the "gold standard". We make no claims as to which method provides the most accurate estimate of "true" cardiac index.

There is little doubt from our paper that the two methods are not interchangeable. If clinicians prefer to use the transthoracic impedance estimate of cardiac output to guide treatment, they should be aware that treatment procedures based on thermodilution estimates of cardiac index cannot be used, and the procedures based on transthoracic impedance estimates of cardiac index will have to be derived.

When considering the relative inaccuracies of thermodilution and transthoracic impedance estimates of cardiac index, it should be remembered that, for all the potential inaccuracies of thermodilution, the central theory is sound. In the case of transthoracic bioimpedance, the impedance signal changes in a direction *opposite* to that predicted by the theory on which the monitor is based.

The theory on which the transthoracic impedance method is based does not stand up to critical examination.

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LONG-TERM BACKACHE AFTER EXTRADURAL OR GENERAL ANAESTHESIA FOR MANUAL REMOVAL OF PLACENTA

Sir,—Drs Vickers and May have identified backache as a common problem following childbirth and examined if it is an important sequela of extradural analgesia performed in labour by using a patient questionnaire [1]. They failed to consider other factors which might have a significant association with backache in postpartum patients. The other factors which were identified by MacArthur and colleagues (nulliparity, Asian race and younger maternal age) [2] should have been included in the questionnaire. Heavy physical work, another factor which has been found to have strong correlation with persistent backache after childbirth, should also be included [3]. All these factors should then be included in a multivariate analysis to give a more accurate assessment of the contribution of extradural block in obstetric practice to long term backache.

N. SAH
*Broadgreen Hospital
Liverpool*

1. Vickers RJ, May AE. Long-term backache after extradural or general anaesthesia for manual removal of placenta: preliminary report. *British Journal of Anaesthesia* 1993; 70: 214–215.
2. MacArthur C, Knox EG, Lewis M, Crawford JS. Epidural anaesthesia and longterm backache after childbirth. *British Medical Journal* 1990; 301: 9–12.
3. Ostgaard HC, Andersson GB. Postpartum low-back pain. *Spine* 1992; 17: 53–55.

Sir,—We thank Dr Sah for responding to our recent article. We were fully aware of the limitations of the study and the many other factors that contribute to backache. However, the number of patients we studied was small, but produced such a wide difference in the incidence of backache between the general anaesthesia and extradural groups that we felt that the preliminary study was worthwhile publishing. Clearly, the Journal's expert assessors agreed also that a preliminary communication was sensible.

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POST-SPINAL HEADACHES IN PARTURIENT WOMEN

Sir,—With reference to the recent article on spinal anaesthesia for Caesarean section [1], we wish to communicate the result of our audit into the occurrence of post dural puncture headache (PDPH) after spinal anaesthesia with the 25-gauge Whitacre needle.

Before 1992, this unit used the 26-gauge Quincke needle, and the average incidence of PDPH was 14% (range 8–17%). For the past 12 months, sole use of the 25-gauge Whitacre spinal needle was implemented. Spinal anaesthesia was administered to a total of 210 women, 73% (154) of whom underwent Caesarean section, with either a single injection technique or a combined spinal-through-extradural technique; the remaining 27% (56) of spinal anaesthetics were single injection techniques for procedures such as manual removal of placenta, forceps delivery or cervical cerclage.

All patients were assessed 24 h after insertion of the spinal anaesthesia for mobility, headache on ambulation, ability to void urine and resolution of the sensory and motor block. All patients were advised to contact the anaesthetist if they developed a headache after their discharge from hospital.

We were pleasantly surprised to have no documented headaches in the 210 spinal anaesthetics performed. This result supports the findings of the Bristol study with regard to the absence of PDPH associated with the use of the 25-gauge Whitacre needle in parturient women.

An advantage of the 25-gauge needle compared with the finer gauge needles is the greater rapidity with which cerebrospinal fluid appears in the hub of the needle, therefore reducing the potential hazard of unrecognized, and therefore repeated dural puncture. It would also appear that the "pencil point" of the Whitacre needle, which is claimed to separate rather than cut the fibres of the dura, is an advantage compared with the construction of the Quincke needle, whatever the gauge.

It now seems, therefore, that the use of the 25-gauge Whitacre needle makes spinal anaesthesia an acceptable technique as regards the low incidence of PDPH in parturient women, although continued audit of its use is necessary over a long period of time, with larger numbers of patients, to confirm this.

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F. CARLI
*Northwick Park Hospital
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1. Shutt LE, Valentine SJ, Wee MYK, Page RJ, Rosser AA, Thomas TA. Spinal anaesthesia for Caesarean section: comparison of 22-gauge and 25-gauge Whitacre needles with 26-gauge Quincke needle. *British Journal of Anaesthesia* 1992; 69: 589–594.
2. Snyder GE, Person DL, Flor CE, Wilden RT. Headache in obstetrical patients: comparison of Whitacre needle versus Quincke needle. *Anesthesiology* 1989; 71: A860.

KINKED INNER TUBE OF COAXIAL BREATHING SYSTEM

Sir,—Further to recent correspondence on inner tube disconnections and leaks in Mapleson D coaxial breathing systems [1] I wish to report on a further problem encountered with this type of system.

A new system was attached to the anaesthetic machine. On routine testing it was clear that the fresh gas flow was obstructed. Close inspection showed the reason. The kink is seen clearly in figure 1, which was produced using transillumination of the distal end of the circuit.

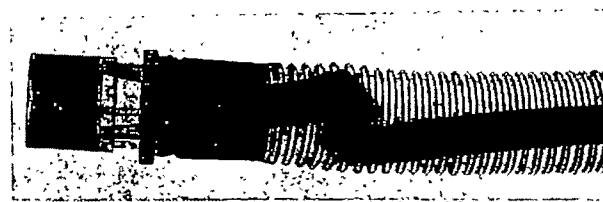


FIG. 1. Kinked inner tube of a coaxial breathing system.

This occurrence again demonstrates the importance of checking anaesthetic equipment before use.

F. P. F. MARSHALL
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Exeter*

1. Robinson DL. Frequency of damaged coaxial breathing systems. *British Journal of Anaesthesia* 1993; 70: 384.

TRANSFUSION FOR WAR WOUNDED

Sir,—Eshaya-Chauvin and I published the transfusion requirements of war wounded in your journal [1]. In this article, we stated that victims of antipersonnel mine require 103.2 units of blood per 100 patients; this is more than for patients injured by other conventional weapons. Since the preparation of this article, Korver and I have found that pattern 1 mine injuries (usually requiring surgical amputation) have even greater requirements [2].

The ICRC database for war wounded now contains information on 12895 patients, 2706 of whom were mine injured. Of the mine injured, those who required surgical amputation of a lower limb required 304.6 units per 100 patients. Your readers who are interested in planning a transfusion service for war injured should recognize the implications of buried antipersonnel mines for the volume of blood required.

R. M. COUPLAND

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1. Eshaya-Chauvin B, Coupland RM. Transfusion requirements for the management of war injured: the experience of the International Committee of the Red Cross. *British Journal of Anaesthesia* 1992; 68: 221-223.
2. Coupland RM, Korver A. Injuries from antipersonnel mines: the experience of the International Committee of the Red Cross. *British Medical Journal* 1991; 303: 1509-1512.

THE REINFORCED LARYNGEAL MASK AIRWAY IN
PAEDIATRIC RADIOTHERAPY

Sir,—We were interested in the article by Williams and Bailey [1], comparing the use of the reinforced laryngeal mask airway (RLMA) with tracheal intubation in anaesthesia for adenotonsillectomy in adults and children, and the editorial by Wilson, on the laryngeal mask airway in paediatric practice [2]. We have had recent experience using the RLMA size 2 in children younger than 3 yr undergoing anaesthesia for radiotherapy. Difficulties encountered with insertion because of their extreme flexibility made them unpopular in this department, amongst a group of anaesthetists skilled in the insertion of the laryngeal mask airway (LMA) in children.

The development of the LMA has transformed anaesthesia for paediatric radiotherapy when young children require repeated daily anaesthetics in an isolated environment. The LMA, sizes 1 and 2, have been used in more than 2500 anaesthetics given to 145 children over the past 4 years in our radiotherapy department and there have been minimal complications associated with their use [3].

Problems arise when treatments require the child's head to be placed within a rigid shell, sometimes in the prone position. Flexion of the neck occurs and kinking of the LMA size 2 at the level of the oropharynx is frequently observed on x-ray. In a retrospective survey of x-rays taken during the planning of treatment in shells, of 15 sets of films retrieved, 11 showed significantly kinked LMA (mean occlusion of lumen in lateral view was 60%, range 25-85%). In none of these patients was there any clinical evidence of airway obstruction, but clearly the potential for complete occlusion of the airway existed. Similar kinking has been observed in the LMA during CT scans of children [4]. To overcome this problem, we were keen to use the RLMA.

Because of the extreme flexibility of the RLMA, the manufacturer's recommended technique of insertion involves the use of the anaesthetist's index finger, wedged between the cuff and the tube, to guide the RLMA into the oropharynx, with final placement using a push on the tube from outside the mouth. We encountered problems with this technique. First, the child's mouth often was too small to admit the anaesthetist's finger far enough. Second, even the smallest amount of saliva or lubricating gel rendered the RLMA too slippery against the tip of the finger (there were 13 insertions using this technique: two were easy, 11 were difficult and two attempts failed completely).

To try and overcome these problems, the use of an introducer to stiffen the RLMA was discussed with Colgate Medical. We used a modified gum elastic bougie cut in length so that distally it did not protrude beyond the vents in the mask, and proximally it was hooked over the connector of the RLMA. This created a

stiffness in the tube of the RLMA similar to that in the LMA. The same insertion technique as for the LMA was then applied. This made insertion much easier, although there were still problems because of rotation of the RLMA around the introducer (of 14 insertions, 11 were easy and three awkward). Two x-rays taken during treatment of children lying prone in head shells showed the RLMA size 2 in the correct position and not kinked. In another child, there was an episode of airway obstruction with an RLMA when the child was turned prone (no x-ray was taken during this case).

Williams and Bailey did not specify their technique of insertion of the RLMA, so we assume that they followed the manufacturer's guidelines. Our attention was drawn to the two failed placements of the RLMA in their study, both in children, one of whom was the youngest in the study (3 yr; 15 kg). Insertion was hindered by large tonsils and the flexibility of the RLMA. The seven children in whom the RLMA was used in our hospital were all younger than 3 yr (mean 1 yr 8 months; range 8 months to 2 yr 10 months).

We recommend that a specific introducer be developed to facilitate insertion of the RLMA in small children. This equipment clearly has great potential for use in paediatric radiotherapy and CT scanning, as well as for ENT and dental anaesthesia.

S. L. MOYLAN

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1. Williams PJ, Bailey PM. Comparison of the reinforced laryngeal mask airway and tracheal intubation for adenotonsillectomy. *British Journal of Anaesthesia* 1993; 70: 30-33.
2. Wilson IG. The laryngeal mask airway in paediatric practice. *British Journal of Anaesthesia* 1993; 70: 124-125.
3. Grebenick CR, Ferguson C, White A. The laryngeal mask airway in pediatric radiotherapy. *Anesthesiology* 1990; 72: 474-477.
4. Goldberg PL, Evans PF. Kinking of the laryngeal mask airway in two children. *Anaesthesia* 1990; 45: 487-488.

Sir,—Thank you for the opportunity to reply to the letter of Drs Moylan and Luce. We did use the insertion technique as described in the manufacturer's instruction manual [1].

To minimize problems with holding the reinforced laryngeal mask airway (RLMA), we premedicate with an antisialogogue and ensure that lubricating gel is not placed on the area to be held.

To aid correct placement of the RLMA, we position the patient with the cervical spine flexed and the atlanto-occipital joint extended. The RLMA is held between the thumb and index finger at the join between tube and mask; it is then put into the mouth and pressed up against the hard palate.

In small children, we find that the RLMA can be advanced into the oropharynx and correctly positioned using the index finger alone. The position of the RLMA can be checked and corrected by manual palpation with the index finger. We have no experience with the RLMA in children younger than 3 yr.

Our two failures to insert the RLMA occurred at the beginning of the study period. With experience, the RLMA is almost as easy to insert as the standard LMA. The use of an introducer to stiffen the tube of the RLMA does not help, as it will not prevent lateral rotation of the mask on the tube.

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P. M. BAILEY

*The Royal National Throat, Nose and Ear Hospital
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1. Brain AIJ. *The Intavent Laryngeal Mask: Instruction Manual*, 2nd Edn, 1991.

USE OF THE LARYNGEAL MASK AIRWAY IN CHILDREN
WITH LARYNGOTRACHEAL PATHOLOGY

Sir,—We read with interest Wilson's excellent editorial on the laryngeal mask airway (LMA) in paediatric practice [1]. We question, however, his comments about the usefulness of the

LMA in patients with laryngeal pathology and, in particular, laryngomalacia.

Whilst the LMA may not be the preferred form of airway management for diagnostic laryngoscopy, it may still prove useful in both the management and diagnosis of laryngeal pathology. This was recently illustrated by Smith, Whittet and Heyworth, who reported a 5-month-old infant undergoing diagnostic laryngobronchoscopy for intermittent stridor [2]. During gaseous induction, stridor worsened considerably and the oxygen saturation decreased. A steady state of light anaesthesia was reached, but with an oxygen saturation of 75–80%. A size 2 LMA was inserted easily and a clear airway obtained. A fiberoptic scope was subsequently passed down the LMA and the classical features of laryngomalacia demonstrated. On the basis of this, it was felt that the LMA may be of specific value in laryngomalacia. The successful use of the LMA in tracheal stenosis has also been reported in a child in whom it provided an adequate airway without traumatizing the trachea or increasing airway resistance [3].

Children with laryngotracheal pathology require meticulous airway management which is best performed by experienced paediatric anaesthetists. Although it is possible that the LMA might worsen some forms of laryngeal pathology, we feel that the LMA may prove useful in some patients, particularly those in whom other forms of airway management have failed. As with many other areas of LMA usage in paediatric practice, further studies are required to help define its role. Meanwhile, we would recommend that the LMA is available when anaesthetizing children with laryngotracheal pathology.

J. BRIMACOMBE
A. BERRY
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Perth, Australia

3. Asai T, Fujise K, Uchida M. Use of the laryngeal mask in a child with tracheal stenosis. *Anesthesiology* 1992; 75: 903–904.

Sir,—My caution was directed mainly at the use of the LMA for diagnosis, rather than management of patients with laryngeal pathology. A correctly sited LMA does not permit inspection of the epiglottis with a fibroscope.

The management of the patient described by Smith, Whittet and Heyworth [1] was obviously helped by use of the LMA. This may or may not have been a result of a specific effect of the LMA on lax supraglottic structures. One should not conclude that the LMA is especially helpful in the management of patients with laryngomalacia on the basis of one case report.

I reiterate my comments about insertion technique. Insertion of an LMA with air in the cuff, or carelessly performed, may readily downfold an abnormal epiglottis. This hypothesis is reinforced by a recent paper from Brimacombe and Berry [2] in which they compared insertion techniques in adults. Briefly, they found the epiglottis to be visible fiberoptically more often when the cuff was partially or fully inflated on insertion than when the recommended technique was used. I urge trainees to read their paper and to learn the technique described in detail in the manufacturer's instruction manual.

I agree that the LMA should be available during the management of children with difficult airways.

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BOOK REVIEWS

Outcome After Anaesthesia and Surgery. Edited by J. M. Desmonts. Published by Baillière Tindall, London. Pp. 695; indexed; illustrated. Price £27.50 (£15 if purchased by subscription to series).

This monograph forms Volume 6/Number 3 of Baillière's *Clinical Anaesthesiology* Series, comprising four issues a year. The past 10 years has seen tremendous interest in identifying and assessing morbidity and mortality after anaesthesia or, as some persist in terming them, *adverse outcomes* or *negative outcomes*. The problems of interpreting such data are immense and full of pitfalls. Professor Desmonts from Paris has not only gathered together a superb group of contributors, but has started off the monograph with an interesting personal overview of the epidemiological aspects involved. His gives a succinct analysis of the difficulties presented and quotes published studies from several countries.

Dr Keenan from Richmond, Virginia addresses the outcome and prevention of anaesthetic mishaps—how I wish the phrase *anaesthetic accidents* was still used! This is a concise chapter, given the vast amount of material now available, and he presents the results of the ASA Closed Claim Studies, data from California malpractice suits, studies of critical incidents and data on cardiac arrest and death. He concludes that a real improvement has occurred in the past 10 years.

Patients with coronary artery disease who require some type of surgery, but unrelated to the heart, form a sizeable proportion of most anaesthetists' routine work. Perioperative cardiac morbidity, manifested in several ways, is the leading cause of death in this group of patients. Dr Sebastien Reiz is one of the authors of this section and renowned for his views on the adverse effects of isoflurane through coronary blood flow "steal". The controversy is now put into perspective by the conclusion that isoflurane usually produces significant coronary artery vasodilatation in man only when the inhaled dose exceeds 1-1.5% and there is little risk of vasodilatation unless isoflurane is used as the sole anaesthetic. A complementary chapter discusses the outcome after anaesthesia for cardiac surgery in adults. In these patients, most determinants of mortality and morbidity are related to the surgery itself, with the role of anaesthesia being modest. However, a major initiative is necessary if the incidence of neurological complications following these operations is to be reduced.

Anaesthetists and surgeons are often reminded to weigh up the relative risks and benefits before subjecting a patient to transfusion, but they often have few data to go on. Dr McClelland, Director of Transfusion Medicine in Edinburgh, covers one aspect of the subject—the range of complications of transfusing blood components. He provides data and advice for sound and defensible decisions to be made.

The review by Drs Deborah Browne and Maldwyn Morgan from London, who write on outcome after anaesthesia for labour and operative delivery, is impressive: sound advice is given on the problems implicated and is essential reading for anyone who is involved, or likely to be involved, in obstetric anaesthesia. The two reviews of outcome after outpatient anaesthesia and outcome in geriatric patients, which follow, are again excellent. Both these subjects are growth areas in medicine and already there are indications that, if careful assessment and meticulous delivery of anaesthesia care are ignored, successful outcomes are jeopardized.

The neurological sequelae of open heart operations have been mentioned. Those after regional anaesthesia are separately reviewed; injuries are more likely to be related to improper positioning, or the surgical procedure itself, rather than to the anaesthetic. Nevertheless, serious problems continue to occur, such as nerve injury, infection, haematoma, etc. A separate chapter is devoted to the psychological and neurological disturbances related to anaesthesia. Here, the patient's fears and anxieties are discussed, then the use of drugs in premedication. The problem of awareness during general anaesthesia is considered, together with postoperative phenomena such as emergence delirium after ketamine, amorous behaviour after propofol, and postanesthetic delirium. The authors conclude with an evaluation of short-term psychomotor or cognitive impairment in

which the effects of drugs and antagonists (such as flumazenil) are discussed.

The book concludes with a splendid chapter from John Eichhorn on the influence of practice standards on anaesthesia outcome. As he says, "standards themselves accomplish nothing. Standards that help provoke, shape and direct thinking and behaviour may accomplish something". He considers safety monitoring, practice standards, the Harvard Standards, the American Society of Anesthesiologists' monitoring standards, and monitoring standards around the world. He contemplates improvement in the outcome of anaesthesia and the problems associated with interpretation. However, one cannot get away from the fact that in the United States malpractice premiums specifically for anesthesiologists have been reduced and the perception of the specialty being of very high risk has been reduced over the last few years. This is indirect, but powerful, evidence of a significant decrease in serious anaesthetic accidents. Eichhorn sensibly counsels that it is over simplistic to believe that monitoring standards themselves reduce anaesthesia accidents. The standards are the tangible outward reflection of changes in attitudes and practices partly brought about by improvements in the quality of doctors entering anaesthesia, training programmes, continuing education, research, anaesthetic equipment and risk management initiatives.

These are the heady days of audit and risk management. Much of the former will wither on the vine, but the material in this volume will endure. The whole book is extremely well written, it is understandable and a joy to read. It contains essential important information which should be in the hands of every practising anaesthetist.

A. P. Adams

Congrès National d'Anesthésie et de Réanimation: Conférences d'Actualisation 1992. By the Société Française d'Anesthésie et de Réanimation. Published by Masson, Paris. Pp. 812; indexed; illustrated. Price FFfr. 450.

This book has its origin in the 34th Congress of the French Society of Anesthesiologists. It is the French equivalent of the ASA refresher courses.

There are two main parts to the book each consisting of 26 chapters. The first part deals with a wide range of topics in anaesthesia. Many of the chapters have a familiar ring: most of them are reviews of recent advances in anaesthesia. The second part is a collection of miscellaneous topics devoted to intensive care.

There is considerable variation in quality between the chapters. Many are excellent, with detailed, comprehensive information. There is an abundance of useful, recent references, marred only by spelling mistakes.

The publication of this book is intended to make a permanent record of material presented verbally. This book should be read by all French-speaking anaesthetists (experienced and in training), either to keep up with the recent advances in their specialty or as part of their continuing anaesthetic education.

B. Appadu

Post Operative Critical Care of the Massachusetts General Hospital, 2nd Edn. Edited by W. J. Hoffman and J. D. Wamnick. Published by Little, Brown & Co., Washington. Pp. 506; indexed; illustrated. Price £22.95.

Most consultants discourage the "cook book" approach to medicine, but one of the commonest requests of doctors new to Intensive Care is for some type of practical manual. In reality, most experienced doctors also welcome guidance on areas and techniques outside their usual areas of expertise.

As the title suggests, this book is the "cook book" of the Surgical Intensive Care Unit at the Massachusetts General Hospital and reflects the type of work carried out in this unit. Each

of 30 chapters deals briefly with the theory of techniques and clinical conditions and then proceeds to give detailed guidance on management. There is little to fault in this guidance and most is appropriate and applicable to practice in U.K. hospitals. There is, however, some imbalance in choice of topics—for example, there is a chapter on neurosurgical patients that includes less than half a page on head injury, and a full chapter on management of liver transplant patients, whilst chest drainage has four pages in the Appendix. This imbalance limits the usefulness of the book in the general ICU.

There are relatively few illustrations and the typeface is small, which makes leisurely reading difficult and photoreduction of no doubt useful algorithms such as that on Intermittent Mandatory Ventilation (p.55) renders them virtually illegible. Subject headings at the start of each chapter are particularly useful and the book is comprehensively indexed to help access to information.

Despite these criticisms, I would recommend this book, as it fills a gap in the market. Unfortunately for the editors and publishers, a new British text at a similar price fills the gap rather more effectively for general ICU practice and would have to make this volume a good second choice.

G. S. Routh

Critical Care of the Burned Patient. Edited by L. T. A. Tyllah. Published by Cambridge University Press, Cambridge. Pp. 206; indexed; illustrated. Price £35.00.

Most hospitals in the U.K. receive only a small number of severely burned patients requiring intensive care, as most victims are rapidly transferred to a Burns Unit where essential expertise and facilities are available. This is obviously appropriate for this difficult group of patients who present problems rather different from those of most Intensive Care Patients.

This book gives extensive details of the management of patients in the Burns Unit and the short chapter on Transportation, with its list of essential equipment and drugs, will be the most useful for general readers. Other chapters include assessment, resuscitation, monitoring and care of burns patients, in addition to details of nutritional requirements and surgical management. The chapter on Inhalation Injury is comprehensive and well written and is essential reading. The penultimate chapter on prognosis reminds the reader that skilled management of burns patients has resulted in improved survival rates, but morbidity remains high. A chapter on prevention of burns by improving education and reducing risk factors would be a valuable addition, as it is hoped that such activities will decrease the incidence of this type of injury in future years.

This book would be valuable in all Burns Units and is recommended reading for examination candidates. It is probably too detailed to be in most departmental libraries unless there is a particular interest in the subject.

G. S. Routh

Acute Pain: Mechanisms and Management. Edited by R. S. Sinatra, B. Ginsbury and L. M. Preble. Published by Mosby Year Book Europe Ltd, Aylesford, Kent. Pp. 636; indexed; illustrated. Price £65.50.

This is a large, multi-author American textbook reviewing the complex and important area of acute pain mechanisms and management.

The early chapters review the historical aspects of pain management and explore the reasons why acute pain has been treated and managed poorly in hospitals for years. The next few chapters are dedicated to describing the anatomy, pathophysiology and neurochemistry of acute pain. Throughout all these chapters are very useful summaries of relevant facts, in the form of boxes that help the reader to identify and retain key facts. There are many helpful tables and diagrams that illustrate the text. A consistent feature throughout this book is that each chapter is referenced comprehensively. I found the chapters on pain assessment and measurement useful, with clear explanations.

Patient controlled analgesia (PCA) is covered widely; the history of the development of PCA devices and the concept of PCA are reviewed thoroughly in chapter 19. PCA as an

investigative tool and PCA and post-surgical outcome are very relevant, as is the chapter on the influence of PCA on morbidity and duration of hospital stay. Of particular interest to anaesthetists who may be contemplating buying PCA devices is a review of their desirable features, and a table of the various characteristics of selected pumps is presented. Several commercially available PCA devices are described, with descriptions of the mechanisms of operation and associated photographs of the particular pump.

The next section provides an overview of spinal opioid analgesia, presenting the reader with a review of the postoperative use of spinal and other opioids. The next few chapters consider the use of extradural analgesia, including discussion of some controversial areas including the use of extradural analgesia in patients who have received or will receive anticoagulation. The use of interpleural analgesia is described in some detail, as are peripheral nerve blocks, with detailed descriptions of methods of performing some of the commoner nerve blocks. Interestingly, newer methods of providing acute pain management are covered in the chapters devoted to transdermal delivery of fentanyl and of other opioids.

Alterations in pharmacokinetics and physiological disturbances with failure of one or more major organ systems are described. A discussion of exactly how these alterations impinge upon the provision of acute pain relief in the postoperative period is relevant to many clinical situations.

Paediatric analgesia is discussed separately, with an overview of paediatric analgesia followed by a chapter on parenteral and analgesic therapy and PCA in the paediatric population.

The final chapters of this book are devoted to some of the organizational issues surrounding acute pain management, including chapters on the role of the anaesthetist and the management of acute pain services in a large university-based hospital or in a more community-based setting. Of particular relevance to hospitals in the U.K. where development and expansion of acute pain services are being planned, are the chapters on nursing education and PCA. Finally, and rather topically for practitioners in the U.K., management issues are discussed, including a chapter on Hospital administration and the acute pain service and also a chapter on acute pain management and payment.

In conclusion, this is a large and comprehensive textbook which systematically covers virtually all areas relating to acute pain management. I found this book to be generally well written, and certainly well referenced, allowing the reader to follow up any special areas of interest. I would certainly recommend it to anaesthetists and other interested professionals concerned with the provision of acute pain services.

J. A. Langton

Psychology, Psychiatry and Chronic Pain. Edited by S. P. Tyrer. Published (1992) by Butterworth Heinemann Ltd, Oxford. Pp. 207; indexed; illustrated. Price £35.

Most patients who are referred to pain management clinics have pain with a significant affective component. In many, the major component of the pain may result from the way in which the patient perceives or reacts to the original problem. The greater efficacy of the multidisciplinary approach to these problems of persistent pain and distress is only gradually being recognized and the disciplines of psychology and psychiatry are still poorly represented in the treatment areas of acute medicine and chronic pain. Communication between specialists can be unnecessarily difficult because of different jargons, especially in formal referrals and reports. This book should increase comprehension of the psychological components of patients' pain, their interaction with the structural and functional components and the ways in which psychologists, psychiatrists and others can help in the assessment and management of patients.

The foreword, by Professor Wall, is so distinctive that, from the written page, one can hear the slow, measured delivery of a pithy, provocative and masterly summary. Subsequently, the book is divided into two parts: the first descriptive and mainly theoretical, the second related to assessment and management. The first part is concerned with the theory of pain perception, the prevalence and classification of psychological problems in chronic pain syndromes and the relevance of gain and compensation. The second is the "how to do it" part. It describes the assessment of the problem, including a useful critique of the methods and tools

of assessment. It contains chapters on behavioural techniques, cognitive therapies, the importance of information and the uses of relaxation and hypnosis. It also includes a summary of the use of psychotropic drugs and stimulation analgesia, the use of physiotherapy and an excellent brief description of a multidisciplinary pain clinic and the facilities it can provide.

The book is intended for a wide audience including nurses, psychologists, therapists and clinicians who are involved in pain management, even for the non-medical chronic pain sufferer. The chapters on anatomy, physiology and the use of psychotropic drugs may seem superficial to readers of this journal, but probably provide valuable background to those less expert. Some of the statements in these sections are contentious and references are few. The section on relaxation is over-long and too detailed. The long, monotonous script suggested to induce relaxation and sleep achieves its aim and would be better placed in an appendix at the end of the book.

The preface states that the book is "intended to be free of jargon". This is an unrealistic aim and fortunately so. The explanations of jargon are nearly always in simple terms when it is first used and can only lead to greater understanding and communication. However, in some sections, polysyllabism abounds and I remain unconvinced that the ideas conveyed by "reconceptualisation" and "phenomenology" could not have been expressed in simpler terms.

This is an easily readable book and best read at a few sittings because it forms an integrated review. It also has valuable reference material such as the addresses of training centres of hypnosis and acupuncture and those of agencies which can help the chronically pained or disabled patient. As in any multi-author book, there is some repetition and inconsistency, and more radical editing would have produced a more concise and no less readable book. In some of the practical sections, the ideas and practice reflect those of the Newcastle Pain Clinic, which impart the sense of successful experience rather than that of a theoretical review.

I found it an informative and enlightening book. Most students, doctors in training and established pain clinicians will find it valuable and there can be few experts in multidisciplinary pain management clinics who will not be able to learn something from it. I recommend it not only to every pain management unit, but also to others who routinely manage patients with recurrent or persistent pain, whether or not that pain can be relieved by pharmaceutical or physical methods.

J. I. Alexander

Principles and Practice of Pain Management. Edited by C. A. Warfield. Published by McGraw-Hill Inc. Pp. 552; indexed; illustrated. Price £56.00.

This is a textbook of pain management aimed at the practising pain specialist. It is a comprehensive book covering many key aspects of pain. The book is extremely well referenced throughout. It is multi-authored and while most of the chapters are excellent, some do not present the depth and quality of review that one would expect in a book aimed at the experienced specialist. There is some repetition of topics, but in general the editor has successfully avoided this pitfall. Most of the authors are North American. This, in itself, is not a major criticism; however, the continued use of American proprietary drug names by some authors appears parochial.

The first chapter on historical perspective—although fascinating—is not easy reading and this, unfortunately, distracts from

the content. Thereafter the book is divided into four sections. Part I, entitled "Understanding Pain", comprises four chapters dealing with pathophysiology, pain measurement, psychological aspects and diagnostic measures. The chapter on pathophysiology is a basic review of pain mechanisms. However, knowledge in this area has increased at such a rapid rate, that it is inevitable that new concepts, now well described in journals, are not mentioned. The chapters on pain measurement and psychological aspects are extremely useful reviews of these important topics. The chapter on diagnostic measures concentrates on physical tests—the validity of some of which is now being questioned—and fails to mention the use of practical psychological assessment in the clinic setting.

Part II categorizes pain according to anatomical location. The chapter on headache is an outstanding review of common aetiological causes and prophylactic and symptomatic treatment. Other excellent chapters are those on the topics of neck pain, foot pain and pain in the extremities. The chapter on pelvic pain is written very much from a gynaecological viewpoint. Scant attention is paid to chronic pelvic pain without obvious pathology—a condition commonly seen by the pain specialist. Unfortunately, recent important findings on this difficult problem by clinicians both in North America and Europe are not mentioned. The subsequent chapter on perineal pain is more comprehensive and useful to the practising clinician. Anatomical drawings and the use of tables would have made the chapter on low back pain easier to read. I was surprised that there was no discussion regarding the possible role of facet joints in this condition.

Part III deals with common painful syndromes. An outstanding chapter in this section is that on paediatric pain management. It is only recently that this topic has received the attention that it deserves and one of the authors of this chapter has been a major contributor to the field. It is an excellent chapter, full of practical advice for those dealing with children in pain on both a regular or occasional basis. The difficult pain problems of sympathetic dystrophies, herpes zoster and central pain syndromes are extensively covered in this section. The chapter on medical management of cancer pain fails to mention the "analgesic staircase"—the cornerstone of analgesic prescription. One questions the wisdom of even including discussion of drugs which are not suitable for cancer pain management. The use of U.S. drug trade names in this chapter is particularly irksome.

Part IV—Pain Therapy, is a successful section, the chapters on intraspinal opioid analgesia and extradural steroids both being especially informative and clearly written. TENS placement charts included in the chapter on stimulation induced analgesia will be of immense practical use. The chapter by Aronoff on multidisciplinary pain centres is extremely interesting and discusses establishment of such facilities, accreditation and standard setting. The chapter on assessment of the pain-disabled patient raises many valid points as, increasingly, chronic pain is being recognized as a disability in its own right. Medico-legal considerations are becoming more important in pain management and a chapter on this subject contains much practical and useful advice. The glossary of terms is a useful adjunct to the text. However, I would comment that the definition of allodynia is not that suggested by the International Association for the Study of Pain. The text is not intended as an atlas of regional anaesthesia and although a few of the commonly used blocks are described in a separate section at the end of the book, it should not be purchased for that reason.

This book will be a valuable and comprehensive source of information for the practising pain specialist. It deserves a space in the pain clinic library.

B. J. Collett

EDITORIAL

POSTDURAL PUNCTURE HEADACHE AND EXTRADURAL BLOOD PATCH

The association of postural headache with puncture of the dura mater was known to the early pioneers of spinal anaesthesia. In 1898, August Bier, the "father" of surgical spinal anaesthesia suffered a severe postural headache after an experimental spinal block performed on himself had to be abandoned when a poor fit between syringe and needle resulted in considerable loss of cerebrospinal fluid (CSF). Nearly 100 years later, the exact pathophysiology of the symptoms is still uncertain, although it is known that loss of spinal CSF depletes the intracranial CSF "cushion" of the brain. Movement of the brain in the upright posture then causes traction on pain-sensitive intracranial structures. Pain arising on or above the tentorium cerebelli is transmitted via the trigeminal nerve to the frontal region; pain from below the tentorium is transmitted via the vagus and upper cervical nerves to the occipital region and neck.

Whatever the exact cause of the pain, the cure is to seal the hole in the dura. This always occurs naturally, usually within 1 week, but it may occasionally take much longer. A remarkable (and unwelcome) feature of the postdural puncture headache (PDPH) syndrome is that such a trivial injury may cause such an incapacitating condition. The postural nature of the symptoms makes them particularly disabling and, as a result, attempts to find a cure have been numerous, unsuccessful and at times ill-advised. Tourtellotte listed alphabetically 49 methods of treatment, from abdominal binders (for which at least there was some physiological basis) to x-radiography of the skull (for which there was not). Nelson attempted the ingenious method of passing a length of catgut down the offending needle to seal the hole, but unfortunately this sometimes resulted in cauda equina lesions [1]. Brown and Jones [2] and Harrington, Tyler and Welch [3], as a last resort (but fortunately successfully), carried out laminectomies to close, with clips or a fascial graft, dural holes which were respectively 5 months and 5 years old! Rather more success—although not invariable—has been claimed for the use of extradural saline boluses [4, 5] or infusion [6], and for i.v. caffeine sodium benzoate [7, 8].

The first report of the use of autologous blood injected into the extradural space to "patch" the hole in the dura was made by Gormley in 1960 [9], inspired by his impression (since shown to be erroneous) that dural puncture accompanied by a "bloody tap" was less likely to result in PDPH. Using only 2–3 ml of blood, he claimed 100% cure in his series of eight subjects (one of whom was

himself when suffering from PDPH after myelography). The next significant report was by Odzil and Powell who, using a rather complicated technique of injecting 3 ml of clotted blood into the subarachnoid space and during withdrawal of the needle into the extradural space, claimed no case of PDPH in 100 spinal blocks using a 20-gauge needle, compared with a 15% incidence in a control group [10].

In 1970 [11] and 1972 [12], DiGiovanni and colleagues described two series totalling 108 patients administered a blood patch with 5–10 ml of blood with immediate and permanent cure in 90% and relief in another 8% within 24 h. They also performed laboratory experiments in Angora goats showing that injected blood had largely disappeared from the extradural space in 2 weeks. The technique rapidly became more popular, with anaesthetists relieved to be able to offer effective treatment to sufferers from PDPH. The biggest study was a prospective and multi-centre trial organized by the American Society for Obstetric Anesthesia and Perinatology; this consisted of 185 patients, 182 of whom were completely and permanently relieved of PDPH by an average of 10 ml of blood injected 4 days after the onset of symptoms [13].

The success of the method seemed to have been established, but then argument began as to whether or not blood patching should be carried out early, or even prophylactically. Some workers reported very poor success rates with such use [14, 15], while others claimed good results, stating that the essential ingredient for success was to use adequate volumes of blood (15–20 ml) [16–18]. This argument is unresolved, although the possibility that the truth lies somewhere between is suggested by the work of Colonna-Romano and Shapiro, who found that 15 ml of blood given prophylactically reduced the incidence of PDPH from 76.5% to 17.6%—a considerable reduction, but not so spectacularly successful as some reports in which patching was delayed [19].

In the 30 years that blood patching has been performed, there has been conjecture, but very little evidence, on the mode of action and fate of the injected blood. It was assumed that the blood acted as a plug, and exerted a mass effect in the extradural space for an uncertain duration of time during which the body's reparative mechanisms sealed the hole permanently.

DiGiovanni and colleagues had shown that blood clot effectively sealed holes in a plastic bag of saline. Abouleish and colleagues [20], Naulty and Herold

[21] and others had shown that extradural block was successful when performed after blood patching, suggesting no residual adhesions in the extradural space; and there were no long-term neurological sequelae. Hardy showed, by myelography, that the blood was still present as a space-occupying mass 1 week after injection [22], and Szeinfeld and colleagues [23], in a useful (but unlikely to be repeated) study, used radioactively-labelled red blood cells to show that the injected blood spread extensively, mainly cephalad from the site of injection, to the extent of about 1.6 ml per spinal segment. Cook and Watkins-Pitchford made the interesting laboratory discovery that blood clotting was accelerated in the presence of cerebrospinal fluid [24].

Rosenburg and Heavner gained useful information from *in vitro* experiments on the ability of extradural blood patches of 25-gauge and 19-gauge holes in dog dura to resist pressures similar to those in the lumbar subarachnoid space of the human in the sitting position [25]. After 30 min of exposure of the dura to autologous blood, the blood was found to be adherent in thin sheets over and around the hole in the dura, with clotted blood protruding in a band through to the cerebrospinal fluid side of the hole in several subjects. The patches were then effective at resisting pressures up to 40 mm Hg in the 25-gauge group, although rather less effective in resisting pressures in the 19-gauge group.

In the current issue of this Journal, an original and informative study of extradural blood patches using magnetic resonance imaging (MRI) pulls together some of these threads of evidence [26]. Imaging was carried out once each in five patients at intervals of 30 min, 3, 7, 9 and 18 h after blood patching. At 30 min and 3 h, the clot was shown to have a mass effect, compressing the dural sac and displacing the conus medullaris and cauda equina. There was also compression or displacement of nerve roots nearby. The main bulk of the clot occupied four or five vertebral levels with thinner spread cephalad and caudad several vertebrae further. Some blood initially increased the CSF signal, indicating its entry into the subarachnoid space, but by 3 h this had concentrated to a focal clot within the dural sac apparently adherent to the dura. Imaging from 7 h onwards showed that the mass effect had disappeared, with a thinning layer of blood adherent to the dural sac, but extending much further cephalad than caudad.

With its images of injected blood initially producing a mass effect resolving to a thinner layer of clot adherent to the posterior aspects of the dural sac, this study confirms earlier assumptions and investigations of the mechanism of extradural blood patch. Temporary displacement of nerve roots corresponds to radicular pain which occasionally accompanies the technique. A surprising feature, not described previously, is the extensive spread of blood back into the subcutaneous fat, and it is suggested that this may be associated with the backache which not infrequently follows blood patching. A tendency for the blood to spread cephalad was noted by Szeinfeld and colleagues [23] and confirms their recommendation that

the same or a lower interspace be used for blood patching.

As the study unfolds, it is easy to forget that (for obvious reasons) the five MRI reports are of five different patients. The assumption is made that this is the typical sequence of events which would happen with any individual blood patch and, while probably reasonable, it is still an assumption and for that reason should be confirmed by additional studies.

Lastly, while providing useful information on what happens when extradural blood patch is effective, it gives less clue on what happens when it is not.

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ORIGINAL ARTICLES

MAGNETIC RESONANCE IMAGING OF EXTRADURAL BLOOD PATCHES: APPEARANCES FROM 30 MIN TO 18 H

S. C. BEARDS, A. JACKSON, A. G. GRIFFITHS AND E. L. HORSMAN

SUMMARY

We have used magnetic resonance imaging to examine five patients treated with extradural blood patches for persistent post lumbar puncture headache. Images were obtained between 30 min and 18 h after patching. Extradural blood patch injection produced a focal haematoma mass around the injection site which initially compressed the thecal sac and nerve roots. The main bulk of the extradural clot extended only three to five spinal segments from the injection site, although small amounts of blood spread more distally. Spread from the injection site was principally cephalad. Mass effect was present at 30 min and 3 h, but clot resolution had occurred by 7 h, leaving a thick layer of mature clot over the dorsal part of the thecal sac. Eighteen hours after injection only small widely distributed clots, adherent to the thecal sac, were demonstrated. Extensive leakage of blood from the injection site into the subcutaneous tissues was present in all patients. (Br. J. Anaesth. 1993; 71: 182–188)

KEY WORDS

Anaesthetic techniques: blood patch, extradural. Complications: post lumbar puncture headache.

Extradural blood patch is the treatment of choice for post lumbar puncture headache (PLPH) occurring as a complication of spinal or extradural anaesthesia [1–3]. The mechanism of action of the blood patch is incompletely understood, despite extensive clinical studies [2, 4–6]. We have recently presented the findings in a single patient investigated by magnetic resonance imaging (MRI) 30 min after injection of 20 ml of blood into the extradural space [7]. MRI demonstrated considerable mass effect from the extradural haematoma, with compression of the thecal sac and of exiting nerve roots. We have now had the opportunity to examine an additional four patients between 3 h and 18 h after patching. The findings from all five of these patients are presented here and include new observations on the original patient which were appreciated only after examination of later subjects.

PATIENTS AND METHODS

We studied five patients with severe PLPH resulting from accidental dural tap during extradural anaesthesia (two patients), after spinal anaesthesia (one

patient) and after combined extradural and spinal anaesthesia (two patients). In the two patients with combined extradural and spinal punctures, there was no evidence of dural puncture by the Tuohy needle.

After local Ethics Committee approval, informed consent for MRI was obtained from each patient.

Extradural blood patches were performed after 24–84 h of unsuccessful conservative therapy (table 1). Patching was performed with the patient in the sitting position. The technique of loss of resistance to air was used to locate the extradural space. All patches were performed using a 16-gauge Tuohy needle to inject 18–20 ml of autologous venous blood at the level of or adjacent to the original extradural puncture. Patches were performed by a senior registrar (three patients) or consultant in obstetric anaesthesia (two patients).

The dorsolumbar spine was imaged in each patient at a pre-determined interval (30 min–18 h) after patching. Imaging was performed using a 0.5 Tesla Max System (General Electric). Each patient was

TABLE 1. Basic clinical data for all patients. In patients Nos 1 and 4, two levels are indicated for the original procedure. In each, attempted extradural injection at the upper level was complicated by dural puncture. Successful extradural anaesthesia was subsequently established at the lower level

Patient No.	Age (yr)	Weight (kg)	Procedure and level	Duration of headache (h)	Patch level	Time to MRI (h)
1	35	78	Extradural L2–3 + L3–4	36	L1–2	0.5
2	29	65	Spinal L2–3	36	T12–L1	3
3	44	81	Spinal + extradural L3–4	24	L2–3	7
4	25	130	Extradural L1–2 + L2–3	84	T12–L1	9
5	24	93	Spinal + extradural L2–3	72	L2–3	18

This article is accompanied by an Editorial.

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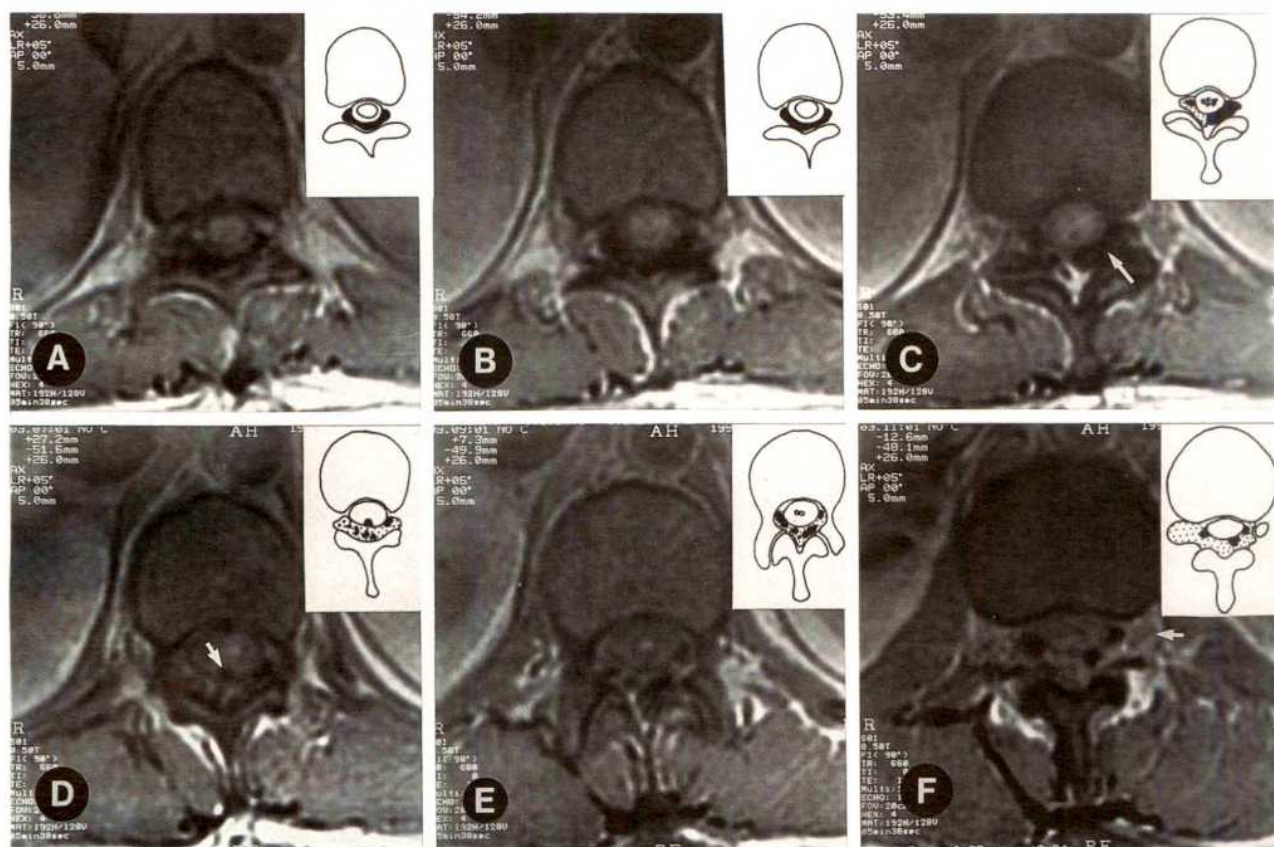


FIG. 1. A series of six axial T1 weighted GE images, demonstrating the distribution of blood within the extradural space in patient No. 2 (3 h after injection). The inset on each image shows the distribution of blood derived from both axial T1 and sagittal STIR images. Images are equidistantly spaced between T10 (A) and L3 (F). The arrow on c indicates clot extending out through the left neural outlet foramen. In D, the arrow indicates a small focus of clot lying within and adherent to the thecal sac. The arrow in F indicates the dorsal root ganglion displaced downwards and anteriorly by the extradural clot.

imaged in the sagittal plane using a T1 weighted gradient echo sequence (GE 300-480/14/90.8 Nex, 96*256 matrix, 42-cm field of view). The use of a large field of view allowed visualization of the extent of haematoma from the T6-7 vertebrae to the sacral recess. Axial T1 weighted images were obtained also throughout the visualized haematoma (GE 480-670/14/90.4 Nex, 192*128 matrix, 22-cm field of view) in all patients. In four patients additional sagittal plane images were obtained using a Short Tau Inversion Recovery (STIR) T1 weighted sequence (IR 1500/110, 4 Nex, 96*224-256 matrix, 42-cm field of view) to suppress the signal from extradural and subcutaneous fat.

Interpretation of MRI images

MRI provides a highly specific and sensitive demonstration of clotting blood [8]. During the first 2-10 h of clot development, haematoma appears as a markedly reduced signal on T1 weighted images. The presence of abundant fat within the extradural space provides clear contrast with larger areas of developing clot, but obscures smaller areas by swamping them with surrounding high signal. The use of STIR images specifically suppresses the high signal of fat and sensitively demonstrates fresh clot as areas of extreme high signal. The combination of axial T1 GE and sagittal STIR images allows demonstration of both the overall extent (STIR images) and the areas of greatest concentration (T1

GE images) of blood within the fat-containing extradural space. Delineation of haematoma from CSF is not a problem with each sequence, as the paramagnetic effect of the clot results in a signal intensity less than that of CSF on T1 weighted images and greater than that of CSF on T2 weighted images.

RESULTS

Clinical findings

All patients were suffering from severe PLPH at the time of patching. In patients Nos 4 and 5, potential technical problems caused by obesity led to a prolonged trial of conservative therapy before patching. There was a dramatic response to blood patch injection, with rapid relief of headache and postural symptoms within 30 min in all patients. There were no late recurrences of headache and repeat patching was not required in any patient. Three patients (Nos 1-3) complained of moderate local backache at the puncture site after patching; this resolved within 24 h in each.

Magnetic resonance findings

Patient No. 1, 30 min after patching. Imaging findings in this patient have been presented previously [7]. Parasagittal images demonstrated a sizeable blood clot in the posterior extradural space. The clot extended upwards from the puncture site to the T11-12 intervertebral space and down one

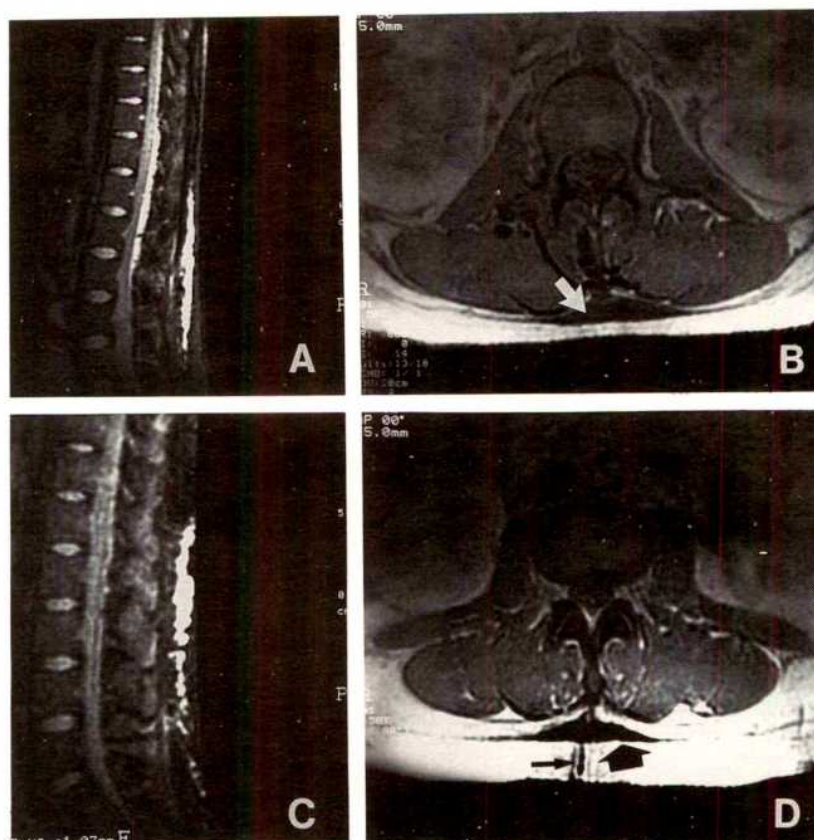


FIG. 2. A: Sagittal STIR image in patient No. 2 (3 h after injection), showing the distribution of extradural blood and extensive subcutaneous spread (clot appears white on this image). B: Axial T1 image in patient No. 2, showing extension of blood from the injection site into the fascial layers of the subcutaneous tissues (arrow; blood appears dark on this image). C: Sagittal STIR image in patient No. 5 (18 h after injection), showing a thin residual layer of extradural blood and extensive subcutaneous spread (blood appears white). D: Axial T1 image in patient No. 5, showing blood spread into the subcutaneous fat (large arrow; blood appears dark) at the level of the needle track (small arrow).

interspace to the L2–3 level. The clot was largest at the levels of the intervertebral discs where the sagittal diameter of the spinal canal is greatest. The clot was seen to compress the thecal sac and displace the conus medullaris and cauda equina anteriorly and to the right. Axial images demonstrated clot extending out through the intervertebral foramina at T11–12, T12–L1 and L1–2 with anterior displacement of the exiting nerve roots. In addition to the focal clot at the injection site, a thin layer of clot extended down the posterior aspect of the thecal sac to the level of the S1 vertebral body, upwards to the level of T9–10 and anteriorly into the anterior extradural space. An increase in the intensity of CSF signal on GE T1 weighted images indicated spread of blood into the subarachnoid space.

A review of the images in this patient demonstrated extensive spread of blood into the fascial planes in the subcutaneous fat overlying the puncture site. This finding was not appreciated initially and was not described when this case was originally reported.

Patient No. 2, 3 h after patching. Extensive clot was demonstrated extending from T7 to L4, with the main bulk of the clot lying between T10 and L3 (fig. 1). The clot was seen to surround the posterior aspect of the thecal sac and to conform to the outlines of the sac. There was generalized compression and anterior displacement of the thecal sac between T11

and L3. Focal clot was seen lying within the thecal sac at T12–L1 which appeared to be adherent to the theca (fig. 1D): the CSF signal was normal. Clot extended out through the neural outlet foramina at several levels (T11–12 to L2–3) and was seen to displace the nerve root at two levels (fig. 1F).

There was extensive spread of blood from the injection site into the fascial planes of the subcutaneous fat, which was well demonstrated on STIR images (fig. 2).

Patient No. 3, 7 h after patching. A thin layer of clot was demonstrated lying adjacent and adherent to the posterior aspect of the thecal sac (fig. 3). Clot extended from T8 to L4, but was most prominent between T11 and L3. There was no significant displacement of neural structures and no evidence of subarachnoid spread of blood. Extensive haematoma was seen to spread from the injection site into the fascial planes of the subcutaneous fat.

Patient No. 4, 9 h after patching. A thick layer of clot extended from T11 to L2 and closely conformed to the outline of the dural sac. A thinner layer of clot extended from T6 to L3. The clot was closely adherent to the thecal sac, forming a “saddle” of haematoma around the theca (fig. 4). There was no evidence of mass effect or subarachnoid blood and extensive spread of haematoma from the injection site into the fascial planes of the subcutaneous fat was again noted.

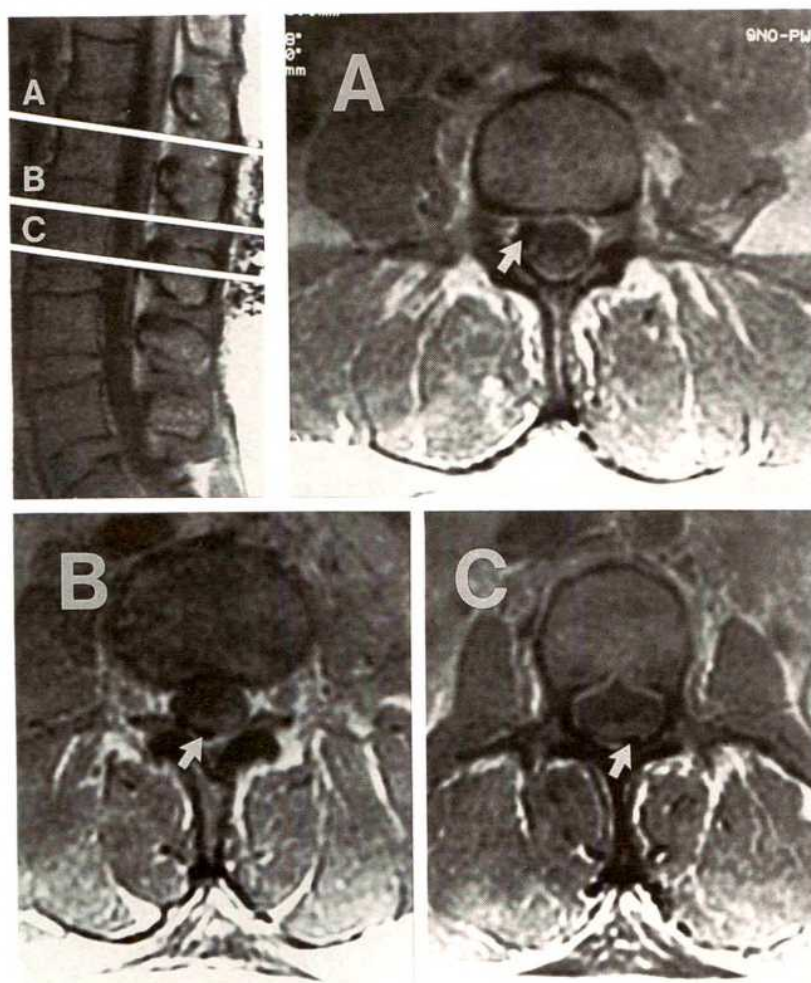


FIG. 3. Distribution of extradural blood in patient No. 3 (7 h after injection). Small foci of clot are seen adherent to the thecal sac at all levels (arrows; blood appears dark).

Patient No. 5, 18 h after patching. Minimal residual clot was seen extending from T12 to L2. The visualized clot was adherent to the thecal sac and had no mass effect. There was no evidence of subarachnoid spread of blood or mass effect. Extensive spread of haematoma into the fascial planes of the subcutaneous fat was again demonstrated (fig. 2).

DISCUSSION

Post lumbar puncture headache is usually a benign, self-limiting condition lasting for only a few days. In severe cases, however, the headache may be totally incapacitating and, without treatment, may last weeks or even months [2, 9].

The mechanism of PLPH is well described [2]. Its incidence and severity reflect the size of the subarachnoid-extradural fistula and are related closely to needle size [10]. PLPH is a particular problem after inadvertent puncture of the dura during attempted extradural anaesthesia. Although this complication occurs only in 1–2% of patients [2, 11] the combination of thecal puncture by a large-gauge Tuohy needle (16–18-gauge) and subsequent labour results in a 75–80% incidence of PLPH [1–3].

Gormley was the first to use injection of autologous blood into the extradural space to repair the thecal

tear [12]. This technique has a high success rate, is relatively free of complications and is the method of choice for treatment of PLPH [2, 8, 13, 14]. The rapid response to patching cannot be explained by simple plugging of the thecal tear, as CSF production (0.5 ml min^{-1}) cannot make up the lost fluid within such a short time [2, 15]. Cook and Watkins-Pitchford demonstrated acceleration of the coagulation pathway in the presence of CSF and suggested that the almost instantaneous coagulation at the blood-CSF interface could explain the rapid therapeutic response [4]. Carrie disagreed with this conclusion, suggesting that the immediate response reflects displacement of CSF into the cranium by the injected volume within the spinal canal [15]. This suggestion is supported by the rapid response to bolus injections of saline into the extradural space [2, 3]. In the current study, the thecal sac was compressed markedly by the extradural clot at both 30 min and 3 h after injection, providing further evidence that this mechanism is responsible for the rapid increase in CSF pressure after blood patch injection. Later images (7 h onwards) demonstrated no residual thecal compression, indicating that this mechanism of pain relief is only short lived. The sustained therapeutic response to blood patches undoubtedly reflects sealing of the thecal tear by clot. A single postmortem examination in a patient

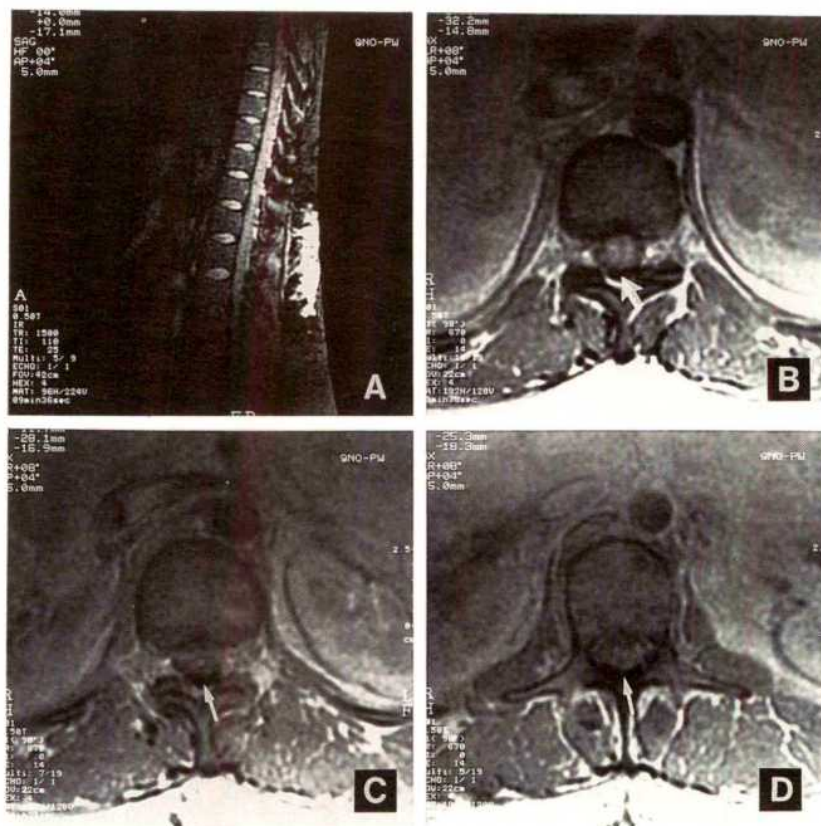


FIG. 4. A: Sagittal STIR image, showing the distribution of extradural blood and subcutaneous spread in patient No. 4 (9 h after injection; blood appears white). B–D: Distribution of extradural blood in patient No. 4. The blood forms a “saddle” of adherent clot along the dorsum of the thecal sac (arrows; blood appears dark).

who received a blood patch 2 days previously demonstrated many separate clots “adherent to the dura like a vulcanising rubber patch” [2]. The appearances in patients Nos 3, 4 and 5 in the current study (figs 2–4) confirm the wide distribution of clot adherent to the theca after resolution of mass effect, particularly in the region of the patch injection. The accelerated coagulation which occurs in the presence of CSF may favour formation of clot around the site of the thecal tear, and the finding of clot extending through the puncture site into the subarachnoid space in patient No. 2 provides an elegant demonstration of the ability of focal extradural blood to seal a thecal tear (fig. 1D). The combination of the initial thecal compression by the extradural clot and the tendency to accelerated clotting in the region of the CSF leak may explain in part why early workers had considerable success with injections of as little as 2 ml of blood [12].

Failure of extradural blood patching is often ascribed to inadequate injection volume [3, 9]. Although Gormley [12], in his original series of seven patients, had excellent results using only 2–3 ml of blood, later workers had failure rates as high as 25% for late blood patch injection using 6–10 ml [2, 6, 14, 16]. Crawford [3, 9] found that the use of increased volumes of blood was associated with a considerable increase in success and recommended routine use of injection volumes of 18–10 ml. Several groups have now examined the relationship between injection volume and success of blood patching and all recent studies have followed

Crawford’s recommendations for large injection volumes [1, 2, 17–19]. In the current study, all patients had a considerable volume of blood extending out from the site of insertion of the Tuohy needle into the fascial planes between the subcutaneous fat compartments. This observation has not been reported previously and raises important questions regarding the fate of the injected blood. It is impossible to estimate accurately the volume of blood within the extradural space in our patients, but it seems clear that it was much less than the overall injected volume. The blood patch injections in our patients were uncomplicated and it seems likely that this leakage of blood is a common occurrence.

The mechanism of this superficial leakage of blood is unclear. It is of interest that in Gormley’s original report of successful patching with volumes of 2–3 ml [12], extradural puncture was performed using a standard, end-hole, spinal needle and the extradural space was localized by withdrawing the needle after initial subarachnoid spinal puncture. Later studies invariably used 16–18-gauge Tuohy needles and required considerably larger injection volumes. Although we can only speculate on the mechanism whereby blood enters the fascial planes, it seems possible that the use of the loss of resistance technique to locate the extradural space could result in the aperture of the Tuohy needle lying only partly within the extradural space during injection of blood. Alternatively, the presence of a relatively large defect in the ligamentum flavum may predispose to leakage of blood around the needle during injection or after

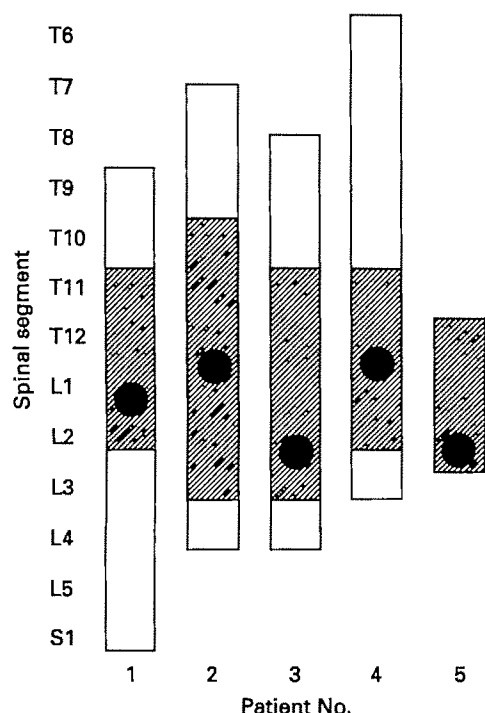


FIG. 5. Graphical demonstration of extradural blood distribution in all five patients. The patch injection level is indicated by a black dot. Hatched areas indicate the extent of clot contiguous with the injection site as demonstrated on T1 weighted GE images. Open boxes show the maximal spread as demonstrated on STIR images.

needle withdrawal. Whatever the mechanism of blood leakage, the current recommendations for optimal injection volumes probably relate poorly to the actual volume of blood injected into the spinal canal.

It is of interest that superficial leakage of blood was not demonstrated in the study of Szeinfeld and co-workers [19]. These workers used a gamma camera to document the spread of technetium-99m labelled blood in the extradural space during and after blood patch injections of 12–18 ml via a 17-gauge Tuohy needle. As no images were presented in this study, it is not possible to comment on whether or not the subcutaneous tissues were included within the collimated field which was examined. It is also of interest that only the first 10 ml of each injection was radiolabelled, so that leakage at the end of the injection may have been overlooked.

Another postulated cause for failure of blood patching is introduction of extradural clot at the wrong level [20]. This might result from alteration in the surface markings of the puncture site after delivery and has also been used to explain the relatively low success rate of extradural patching in some studies in which blood was introduced down the extradural catheter, the tip position being unpredictable [3, 20, 21]. In comparison, the study of Shah and Veness [18] had a success rate of 100% using 15–20 ml of blood injected via an extradural catheter introduced only 2–3 cm beyond the tip of a Tuohy needle. This is perhaps surprising, as the imaging study of Szeinfeld and co-workers [19] demonstrated wide spread of blood within the extradural space. Injections of 12–17 ml in this study resulted in spread over seven to 14 spinal segments

and the extent of spread was directly related to the injection volume, with an average extent of one spinal segment per 1.6 ml of blood. This represents a slightly more extensive spread of blood than was seen in the current study, although this is not unexpected since radiolabelling studies are exquisitely sensitive to even small quantities of tracer and might be expected to demonstrate volumes of blood too small to be seen reliably on MRI [22]. The findings in the current study largely explain the apparent importance of the injection site despite the extensive spread of blood in the extradural space. Although our study demonstrated blood spread over nine to 10 spinal segments after injections of 18–20 ml, the majority of the clot and all the mass effect in patients Nos 1 and 2 was restricted to three to five segments around the injection site, with only small amounts of blood seen more distally. In common with Szeinfeld and co-workers, we found that the spread of clot was principally upwards from the injection site (fig. 5) and would therefore recommend that blood patch injections should be made at the level below the original spinal puncture.

Complications of blood patching are unusual and usually self-limiting. Backache is the most commonly reported complication, occurring in 20–35% of patients, with an average duration of 27 days [1]. The mechanism of backache remains unexplained, but the demonstration of extensive subcutaneous haematoma in the present study may be one causative factor. Short-lived radicular pain is a rarely reported complication [2, 23, 24] and the demonstration of nerve root displacement in patient No. 1 confirms the assumptions of previous workers that this results from nerve root compression by extradural clot. Spread of blood into the subarachnoid space has been suggested as a possible source of late arachnoiditis. Although the present study confirms the presence of subarachnoid spread, reports suggest that there is no reason to believe that this will give rise to late complications [2, 25].

On the basis of our findings, we would recommend that extradural blood patch be performed below the original site of puncture, but within one to two spinal segments. If the technique is performed via a Tuohy needle, gradual injection of up to 20 ml of blood is appropriate and smaller volumes should be used only when injection is limited by patient discomfort.

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NITROUS OXIDE ELIMINATION AND DIFFUSION HYPOXIA DURING NORMO- AND HYPOVENTILATION

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AND J. P. BENGTSON

SUMMARY

We studied the elimination rate of nitrous oxide in 36 patients undergoing orthopaedic surgery. They were allocated randomly to one of six groups which differed in time of nitrous oxide exposure and mode of ventilation. In order to simulate recovery conditions, nitrous oxide administration was discontinued after 30, 60 or 120 min of exposure. Either normoventilation or hypoventilation was used. The mean excretion rate was 1 litre min^{-1} at 1 min, declining to 100 ml min^{-1} at 30 min, with relatively small effects of different modes of ventilation and times of exposure. In spite of an FI_{O_2} of 0.30, there were significant decreases in Sp_{O_2} during both normo- and hypoventilation. The smallest end-tidal oxygen concentrations were reached at 10–15 min in the groups with hypoventilation, after 1 or 2 h of nitrous oxide exposure. (Br. J. Anaesth. 1993; 71: 189–193)

KEY WORDS

Anaesthetics, gases: nitrous oxide. Hypoxia: diffusion.

The time required for the elimination of nitrous oxide from the body during recovery from anaesthesia is predicted to be similar to that required for saturation with the gas [1, 2]; there is a close agreement in relative uptake and excretion curves for nitrous oxide [3, 4]. There are few quantitative studies of nitrous oxide elimination, but in one study, peak nitrous oxide outflow was 1 litre at 1.5 min [5].

The effect of ventilation on end-tidal concentrations has been studied [6], but it is not clear if ventilation has any effect on body elimination of nitrous oxide. In theory, there is no appreciable effect of increasing ventilation if the anaesthetic is as poorly soluble as nitrous oxide [7].

Arterial oxygen desaturation may develop when nitrous oxide–oxygen anaesthesia is discontinued and the patient breathes room air [8]. Because nitrous oxide is more soluble in blood than nitrogen, the excretion of nitrous oxide into the alveoli is greater than the uptake of nitrogen from the alveoli into the blood. The dilution of alveolar oxygen by nitrous oxide may cause arterial hypoxaemia. This diffusion hypoxia is said not to be clinically significant in healthy patients who maintain normoventil-

ation [9, 10]. During controlled normoventilation of patients undergoing neurosurgery, Pa_{O_2} decreased by 2.2 kPa, 10 min after the inspired gas was changed from 79% nitrous oxide in oxygen to air [11]. Normal subjects sedated with 75% nitrous oxide for 90 s during moderate hyperventilation demonstrated arterial desaturation, caused mainly by apnoea [12].

The aim of this investigation was to study nitrous oxide elimination rates during recovery with normo- and hypoventilation after different durations of exposure to nitrous oxide, and to evaluate the influence on end-tidal oxygen concentration and oxygen saturation.

PATIENTS AND METHODS

The study was approved by the Ethics Committee of the University of Göteborg.

A non-rebreathing system with a Servo 900C ventilator was used, with a 1-litre mixing box connected distal to the expiratory valve. Gas for concentration analyses was sampled from the outlet of the mixing box and from the connection between the Y-piece and the tracheal tube. A Capnomac Ultima (Datex Instrumentarium OY, Helsinki, Finland) monitored inspiratory and end-tidal concentrations of nitrous oxide, oxygen, carbon dioxide and isoflurane or enflurane. This gas monitor also intermittently monitored mixed expired gas concentrations. The gas analyses were collected on-line to a computer using a Datex Daisy software program. A Datex Cardiocap was used for pulse oximetry. An Ohmeda 5420 Volume Monitor (Ohmeda, BOC Health Care Division) measured expiratory ventilation volume. This is a turbine vane monitor with a stated tidal volume accuracy of $\pm 8\%$ or $\pm 40 \text{ ml}$, whichever is greater, within the range 200–3000 ml. A heat-moisture exchanger was used for inspiratory gas humidification.

Anaesthesia was induced with thiopentone 4–5 mg/kg body weight, tracheal intubation was facilitated with suxamethonium 1 mg kg^{-1} and pancuronium $0.06\text{--}0.15 \text{ mg kg}^{-1}$ was used for

TABLE I. Patient data (mean (range))

	Group					
	N-30	H-30	N-60	H-60	N-120	H-120
Weight (kg)	80 (56–95)	75 (58–94)	80 (65–91)	69 (56–81)	68 (43–93)	67 (52–80)
Height (cm)	176 (168–182)	175 (158–194)	180 (176–183)	170 (159–181)	173 (160–190)	172 (159–188)
Age (yr)	45 (17–81)	36 (15–73)	54 (23–76)	40 (15–69)	52 (18–77)	37 (22–50)
Sex (M/F)	4/2	4/2	6/0	3/3	3/3	1/5

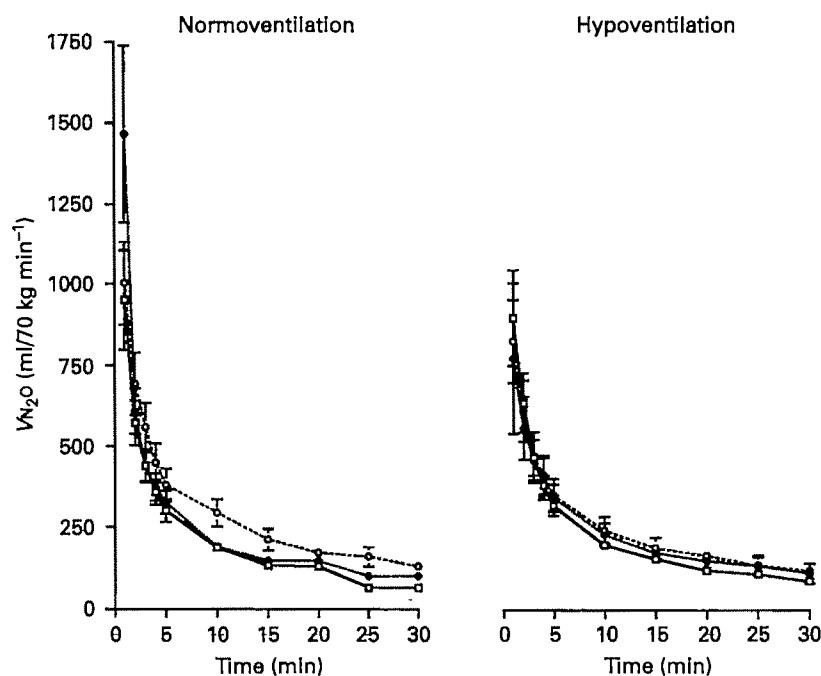


FIG. 1. Mean (SEM) nitrous oxide excretion rates ($\dot{V}_{N_2O}$) during normoventilation and hypoventilation after nitrous oxide exposure of 30 ($\square$), 60 ($\blacklozenge$) or 120 ($\circ$) min.

neuromuscular block. Fentanyl (total dose 0.002–0.006 mg kg⁻¹) was used at induction and during anaesthesia, to provide analgesia. Inspiratory concentrations of 30% oxygen, < 2% enflurane or isoflurane, and 68–70% nitrous oxide were used.

Nitrous oxide was discontinued during the anaesthetic in order to simulate postanaesthetic conditions, after the patients had received it for 30, 60 or 120 min. Then air and oxygen were used, with the inspiratory oxygen concentration unchanged at 30%. The inhalation agent vaporizer setting was also unchanged during the subsequent 30-min study period. To substitute for the anaesthetic effect of nitrous oxide, midazolam 0.04 mg kg⁻¹ was given i.v. 5 min before cessation of nitrous oxide administration.

During the period of nitrous oxide elimination, either normoventilation or hypoventilation was maintained: the inspiratory ventilation volume used to maintain an end-tidal carbon dioxide concentration (E'_{CO_2}) of 4.0% was either kept constant or reduced by 50% 5 min before the discontinuation of nitrous oxide and then kept constant during the following 30-min study period. Ventilatory frequency was unchanged, at 14 b.p.m. If Sp_{O_2} decreased to less than 90%, the inspiratory oxygen concentration was increased by 5% increments until oxygen saturation reached 90% or more.

After obtaining informed consent, we studied 36 ASA I or II patients undergoing orthopaedic surgery with an expected anaesthesia duration of at least 2.5 h. Patients were allocated randomly to one of six groups:

Group N-30. Patients were given nitrous oxide (FI_{N_2O} 0.68–0.70) for 30 min. The inspiratory ventilation volume used to maintain E'_{CO_2} 4.0% at 30 min was kept constant during the following 30-min study period.

Group H-30. Patients were given nitrous oxide for 30 min, as in group N-30. The inspiratory ventilation used to maintain E'_{CO_2} 4.0% at 25 min was reduced by 50% 5 min before the nitrous oxide elimination period. Ventilatory frequency was unchanged, at 14 b.p.m.

Group N-60. Nitrous oxide was given for 60 min and the inspiratory ventilation volume maintaining E'_{CO_2} 4.0% at 60 min was kept constant during the study period.

Group H-60. Nitrous oxide exposure was for 60 min and inspiratory ventilation volume was reduced by 50% 5 min before discontinuation of nitrous oxide, as in group H-30.

Group N-120. Nitrous oxide exposure was for 120 min. Ventilation volumes were as in groups N-30 and N-60.

Group H-120. Nitrous oxide was given for

120 min. Ventilation volumes were as in groups H-30 and H-60.

Two patients, one in group H-60 and one in group H-120, had Sp_{O_2} values less than 90% at 6 and 3 min, respectively. FI_{O_2} was increased to 0.55 and 0.35, respectively, until adequate Sp_{O_2} was obtained. The inspiratory to end-tidal oxygen concentration differences of these patients were extrapolated to FI_{O_2} 0.30, as were the oxygen saturation values.

Statistics

Results are expressed as mean (SEM) or as mean (range) and compared by one-factor ANOVA repeated measures within groups, followed by Fischer's PLSD test. A one-way ANOVA and Fischer's PLSD test were used for comparison between groups. Best-fit curves for nitrous oxide excretion rates were created according to a power model. Statistical significance analysis was performed through the calculation of best fit curves for each patient. The following formula was used:

$$VN_2O (\text{ml}/70 \text{ kg min}^{-1}) = bt^{-m}$$

where b = 1-min elimination rate; t = time after cessation of administration of nitrous oxide (min); m = negative "slope" of the curve.

Statistical significance was assumed for $P < 0.05$.

RESULTS

Patient data are presented in table I. The nitrous oxide excretion rates were normalized by weight to $\text{ml min}^{-1}/70 \text{ kg}$ (fig. 1). The mean excretion rate was 987 ml min^{-1} at 1 min, declining to 103 ml min^{-1} at 30 min. Best fit curves gave significantly greater b and m values in groups N-30 and N-60 compared with groups H-60 and H-120 ($P < 0.05$ in all cases), indicating larger initial excretion rates in groups N-

TABLE II. Best-fit curves created for each patient according to the formula $VN_2O (\text{ml}/70 \text{ kg min}^{-1}) = bt^{-m}$ (mean (SEM))

	Group					
	N-30	H-30	N-60	H-60	N-120	H-120
b	1263	1082	1371	821	908	778
SEM	125	169	106	66	131	65
m	0.76	0.67	0.72	0.57	0.59	0.57
SEM	0.04	0.06	0.04	0.02	0.02	0.03

30 and N-60, but a more prolonged nitrous oxide excretion in groups H-60 and H-120 (table II).

The mean expired ventilation volume was 7.13 litre in the three groups with normoventilation and 3.20 litre in the groups with hypoventilation. E'_{CO_2} decreased gradually in the groups with normoventilation during the 30-min nitrous oxide elimination period. Mean E'_{CO_2} values at 30 min were 3.60 (SEM 0.16)% in group N-30, 3.83 (0.11)% in group N-60 and 3.78 (0.17)% in group N-120. Corresponding values during hypoventilation were 6.75 (0.24)%, 7.35 (0.15)% and 7.40 (0.20)% in groups H-30, H-60 and H-120, respectively.

Mean end-tidal nitrous oxide concentration declined rapidly from 66 to 70% at 0 min to 6–9% at 5 min and to 2–4% at 30 min during normoventilation. In the groups with hypoventilation, the values were 16–23% at 5 min and 5–9% at 30 min (fig. 2).

The maximum inspiratory to end-tidal oxygen concentration difference was 5.7% in group N-30, 6.0% in group N-60 and 6.3% in group N-120, all at 3 min. During hypoventilation, corresponding maximum values were 11.9% at 4 min in group H-30, 14.8% at 10 min in group H-60 and 14.8% at 15 min in group H-120 (fig. 3). The curves were significantly different between normo- and hypoventilation ($P < 0.05$ in all cases), but also between groups H-30 and H-60, and between groups H-30 and H-120 ($P < 0.05$ in both cases).

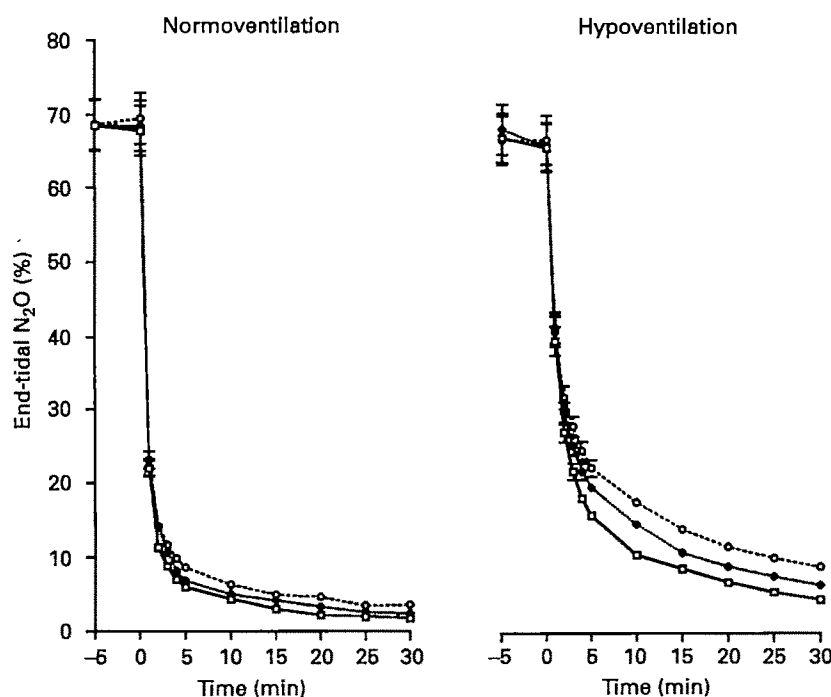


FIG. 2. Mean (SEM) end-tidal nitrous oxide concentration during normoventilation and hypoventilation after nitrous oxide exposure of 30 (□), 60 (◆) or 120 (○) min.

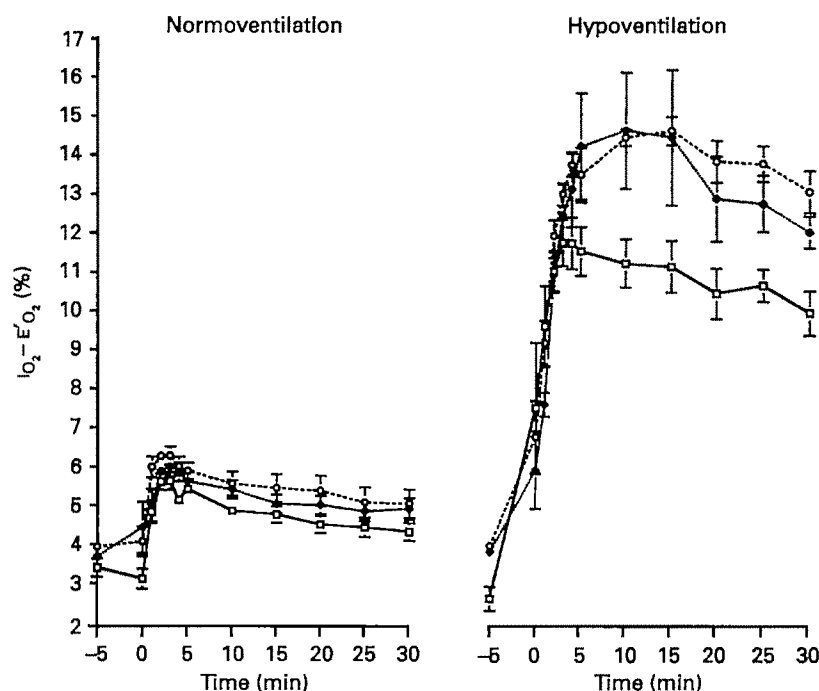


FIG. 3. Mean (SEM) inspiratory to end-tidal oxygen difference ($I_{O_2} - E'O_2$) during normoventilation and hypoventilation after nitrous oxide exposure of 30 ($\square$), 60 ($\blacklozenge$) or 120 ($\circ$) min.

Mean Sp_{O_2} was 96.5–97.5% at the time of cessation of nitrous oxide (fig. 4). The smallest mean values were 96.0% at 15 min in group N-30, 96.5% at 1 min in group N-60, 97.2% at 4 min in group N-120, 96.3% at 3 min in group H-30, 93.8% at 5 min in group H-60 and 93.5% at 4 min in group H-120. The decrease in Sp_{O_2} between 0 min and 15 min was significant in group N-30 ($P < 0.05$). In the groups with hypoventilation, there were significant decreases in Sp_{O_2} between 0 and 4 min in group H-30, between 0 and 3–20 min in group H-60 and between 0 and 3–30 min in group H-120 ($P < 0.05$ in all cases).

DISCUSSION

This study has demonstrated that nitrous oxide is eliminated at a rate similar to that at which it is taken up. The time of exposure, within the range 30–120 min, had no significant influence on excretion rates. This is an indication that the output reservoir formed by the body is finite and almost full after 30 min of nitrous oxide administration. The effect of hypoventilation on nitrous oxide elimination was most pronounced during the first few minutes, which can be interpreted as a slower elimination from the lung volume of the patient. The ventilatory volumes had little effect on the excretion rates after 5 min. Thus the greatest impact of augmenting ventilation on nitrous oxide elimination is achieved during the first 5 min.

The prerequisites for diffusion hypoxia were present after the first 3–5 min of recovery, which has been suggested previously [7]. The mean inspiratory to end-tidal oxygen concentration difference during hypoventilation, after 1–2 h of exposure to nitrous oxide, reached a maximum of almost 15% at 10–15 min. This would correspond to an end-tidal oxygen concentration of 6% during air breathing.

The end-tidal gas concentrations after 1 h of nitrous oxide administration can be used to simulate a period of recovery breathing room air (fig. 5). Normoventilation is crucial to maintain adequate alveolar oxygen concentrations during the first 30 min of recovery. Hypoventilation had little effect on nitrous oxide elimination after about 5 min; in contrast, the end-tidal nitrous oxide concentration was about doubled in the 5–30 min interval during hypoventilation compared with normoventilation. The end-tidal carbon dioxide concentration increased to about 7% during the first 30 min of hypoventilation during recovery. Thus both nitrous oxide and carbon dioxide dilute alveolar oxygen. With air breathing, the decreasing oxygen tension affects the haemoglobin oxygen saturation on the steep part of the oxygen dissociation curve, with resultant arterial desaturation. In figure 5, we have attempted to calculate the arterial oxygen saturation from the oxygen dissociation curve, using the inspiratory to end-tidal oxygen concentration difference in the study [13]. Hypoventilation after anaesthesia without nitrous oxide would decrease the end-tidal oxygen concentration, but to a much smaller extent.

A recent review on mechanisms of postoperative hypoxaemia found diffusion hypoxia during nitrous oxide elimination “unimportant” [14]. Our study conflicts with this view. In spite of an inspiratory oxygen concentration of 30%, we found decreases in Sp_{O_2} during both normo- and hypoventilation.

In clinical practice, we recommend that nitrous oxide is discontinued early in the recovery period, so that the patient is monitored during the first elimination phase. As hyperventilation does not influence the elimination of nitrous oxide to a large extent, and increases the risk of a subsequent hypoventilation during transport to the recovery area, hyperventilation should be avoided. During transport from the operation theatre to the recovery

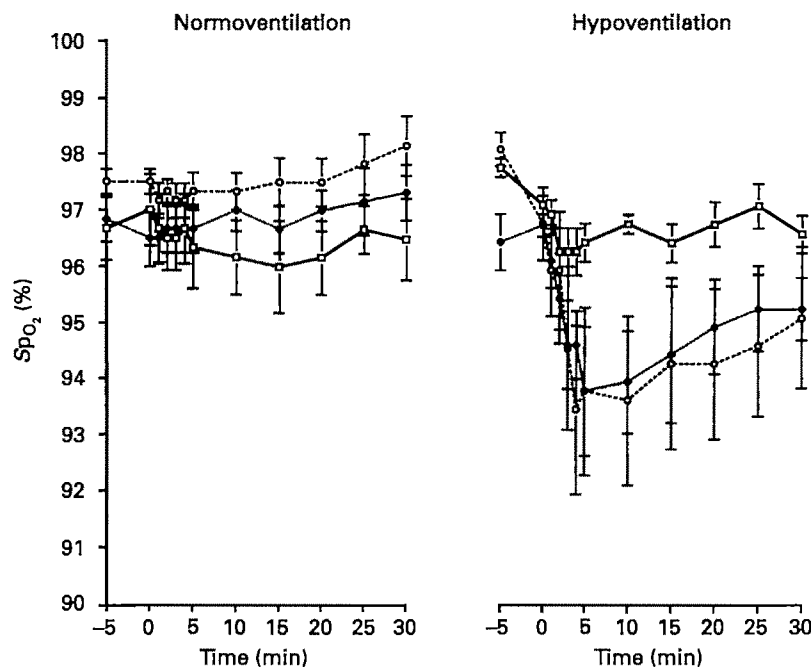


FIG. 4. Mean (SEM) pulse oximetry oxygen saturation (Sp_{O_2}) during normoventilation and hypoventilation after nitrous oxide exposure of 30 ($\square$), 60 ($\blacklozenge$) or 120 ($\circ$) min.

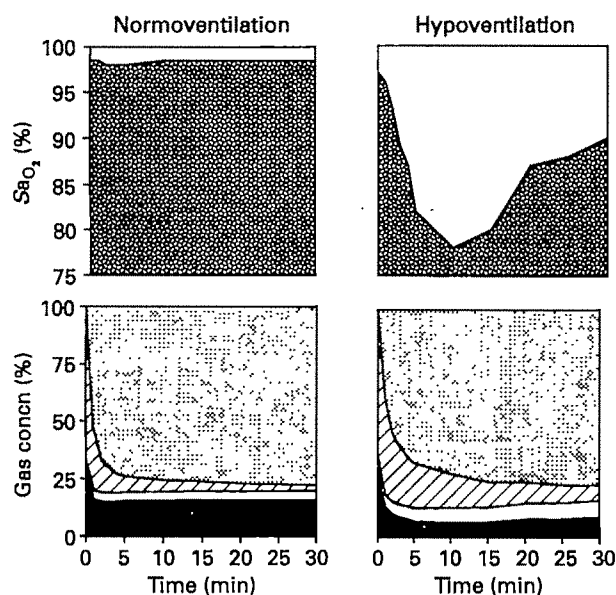


FIG. 5. Calculated arterial oxygen saturation (Sa_{O_2}) (heavy stippling) and alveolar gas concentrations (ATPD) ($\blacksquare$ = oxygen; $\square$ = carbon dioxide; $\square$ = nitrous oxide; $\square$ = nitrogen) during recovery after 1 h of nitrous oxide anaesthesia with normo- and hypoventilation using room air.

room, we strongly recommend the use of oxygen therapy, regardless of the duration of the nitrous oxide anaesthesia.

In conclusion, the nitrous oxide elimination rates were close to those predicted previously, but there is a risk of diffusion hypoxia for at least 30 min from cessation of nitrous oxide administration in the presence of hypoventilation.

ACKNOWLEDGEMENT

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MEASUREMENT OF BREATH-BY-BREATH GAS EXCHANGE DURING GENERAL ANAESTHESIA†

P. C. W. BEATTY, M. J. BEECH AND T. E. J. HEALY

SUMMARY

We describe a single flow transducer breath-by-breath gas exchange measurement system suitable for use during general anaesthesia. The system uses a Fleisch No. 2 pneumotachograph, a mass spectrometer and a microcomputer to give real-time continuous measurements. Correction for apparent gas exchange attributable to changes in gas stored in the lung (functional residual capacity) is available. The correction assumes no gas exchange of either nitrogen or argon for air-breathing subjects or argon only during anaesthesia, while the inspired concentrations are maintained at atmospheric values. The method has been tested against Douglas bag measurement and compared with results from conventional systems used by other authors. The system measurements show broad agreement with Douglas bag measurements, although the limits of agreement are wide for air-breathing volunteers. The system between-breath variation was typical of breath-by-breath methods in other areas of medical research. (Br. J. Anaesth. 1993; 71: 194–200)

KEY WORDS

Lung: function. Measurement techniques: mass spectrometry.

The measurement of gas exchange during anaesthesia and intensive therapy may be used to determine the basic pharmacokinetics of gaseous anaesthetic agents [1, 2], to derive the respiratory quotient as a guide to parenteral feeding regimens [3, 4] or to measure the effects of i.v. drugs [5].

Gas exchange in the clinical environment has been measured using three established techniques. In titration methods, the quantity of gas required to maintain the inspired concentration in a closed environment is measured; these methods have been applied to measurements made during paediatric intensive therapy [6]. Measurements using modified breathing systems have been applied in general anaesthesia [7]. In the investigation of the physiology of exercise, fast measurement response to changes in the work load of exercising subjects is required and the use of breath-by-breath measurement of gas exchange has been favoured [8–10].

The maximum amount of information is obtained usually from breath-by-breath measurement. However, during anaesthesia the use of this method has been limited by the need to correct for the wide

range of gas composition changes encountered. Furthermore, the absence of air nitrogen in the inspired gases prevents the application of Haldane's transformation, in which the ratio of inspired to expired nitrogen concentrations is used to deduce inspired volume from expired volume by assuming no exchange of nitrogen [11]. Additionally, no correction of measurements for changes in the volume of gas stored in the lung attributable to breath-to-breath changes in functional residual capacity (FRC) can be made.

We describe a breath-by-breath gas exchange measurement system which overcomes the problems posed, for eight gases. It uses a single pneumotachograph, a mass spectrometer and a microcomputer. The system is suitable for use during general anaesthesia.

SUBJECTS AND METHODS

Principles of operation

The technical problems faced by any breath-by-breath gas exchange system using a remote sensor of gas concentrations are threefold:

- (1) Correction for the relative delay between flow information and concentration information resulting from lag time in the gas capillary of the measuring instrument.
- (2) The need to correct for different humidity and gas composition between inspired and expired gases and the effect of changes on flow measurement.
- (3) The need to correct flow measurements for the wide range of changes in gas composition within expired gas.

The last two of these problems have often been solved in other reported systems by use of two pneumotachographs calibrated for inspiratory and expiratory gas, respectively. For the system reported here, two pneumotachograph heads would have reduced its applicability in anaesthesia, and so a single flow transducer system was specified. A further design constraint of the system was the need to make measurements in real-time and display the

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†A preliminary account of this study was presented to the Anaesthetic Research Society in Southampton, April 1990 (*British Journal of Anaesthesia* 1990; 65: 276P).

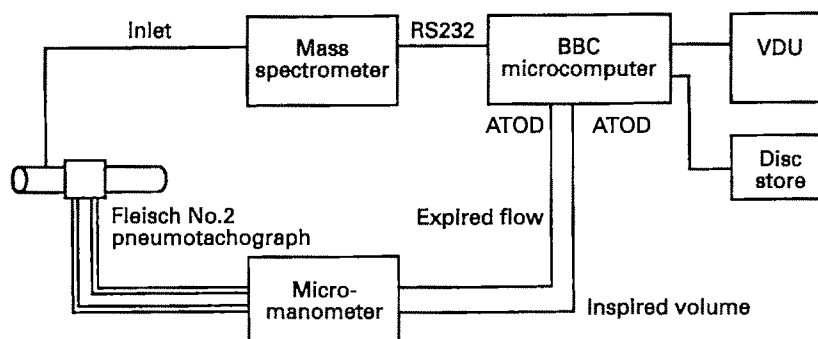


FIG. 1. Schematic diagram of the breath-by-breath gas exchange measurement system.

results breath-by-breath, requiring that results be computed within the time limits of the breathing cycle.

The mathematical basis and the technical details, including error sensitivity analysis of the system and calibration results for the individual components have been reported in detail elsewhere [12, 13]. The design of the system addressed these fundamental problems; design constraints may be summarized as follows:

(1) The delay between the mass spectrometer and the flow transducer was allowed for separately for each breath by identifying the beginning of inspiration in the carbon dioxide concentration channel. This point was associated with the point at which inspiratory flow started on the flow transducer. This method had the advantage that it worked even if the delay changed because of partial blockage of the capillary of the mass spectrometer.

(2) Inspired gases were assumed to be of constant concentration at the concentration values given at the point expiration started on the flow transducer. At this point, the mass spectrometer was still registering inspired concentration, because of its capillary delay time.

(3) Inspired volume of gas was measured by the pneumotachograph micromanometer. This integrated the inspired flow signal on a sample-and-hold circuit, holding it until the next inspiration, when it automatically reset. This eliminated the need to compute inspired volume and gave the computer time for calculation and display in real-time.

(4) Measurement of expired gases flow was corrected for changes in gas composition by assuming that the gases had a constant composition throughout expiration at the mean of the inspired to end-tidal concentration values. It was assumed that the viscosity of the mixture equalled the mean of the viscosities of their components weighted by their concentration. A fixed inspiratory-to-expiratory difference factor was used to allow for humidity.

(5) The mass spectrometer measured the concentration of all gases in the mixture except water vapour, with the result that all gas concentrations were measured as if dry at room temperature.

Because the system relies on a portion of the ventilatory cycle time in which to do its calculation, as ventilatory frequencies increased there came a point at which there was insufficient time for the calculation to be performed. At this point, calculations were performed every other breath. As a

result, the figures produced by the system are not expressed as flow per minute, but as volume per breath. Switching to every other breath occurred in the reported results at approximately 20 b.p.m. and affected around 10% of readings, mostly in the spontaneously ventilating patients and volunteers.

Apparatus

A schematic diagram of the system used is shown in figure 1. Instantaneous gas concentration values obtained by the mass spectrometer (VG Spectralab-M) are passed to the computer via an RS 232C interface. Inspired volume and expired flow signals from the pneumotachograph micromanometer are passed to the same microcomputer using two analog-to-digital converters. The capillary of the mass spectrometer is mounted on the patient side of an assembly including a Fleisch No. 2 pneumotachograph head. The whole assembly can be inserted into any standard anaesthetic circuit at the catheter mount. The sampling rate for the system was 10 Hz.

In all the tests described, the mass spectrometer was calibrated using a static gas mixer [14]. The accuracy of the instrument so calibrated has been described elsewhere [15].

The software run by the BBC microcomputer is menu-driven and stores data collected on a breath-by-breath basis onto floppy disc. Event markers and patient information are stored on the same disc. Breath-by-breath uptake, end-tidal and end-inspiratory concentrations for all the gases monitored, together with a measurement of respiratory quotient (RQ), are available. The display allows one of these calculated functions to be displayed graphically breath-by-breath in 10-min periods, with the other functions being simultaneously displayed numerically.

Gas exchange measurement methods

The mathematical theory underlying the system has been described by several authors [9, 10, 12, 13, 16]. Two forms of breath-by-breath measurement of gas exchange were implemented in the computer program.

In the simplest, no correction for lung gas stores, breath-by-breath, was made. Gas exchange at the mouth was calculated only from the difference between the inspired and expired volumes of each gas. The inspired volume of each gas was calculated from the constant inspired gas concentration and the

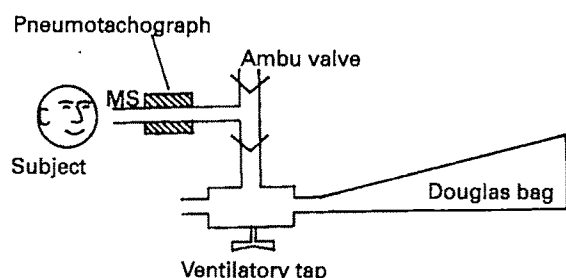


FIG. 2. Experimental arrangement for testing in air-breathing volunteers.

total inspired volume. The expired volume of each gas was calculated from the summation over the expiration of the instantaneous product of gas concentration and volume expired.

In the second method, it was assumed that one of the two inert gases with which the body is saturated from the atmosphere was maintained at atmospheric inspiratory concentration. In these circumstances, there can be no effective concentration gradient between inspired gas and the body and any breath-by-breath gas uptake detected in these gases by the system must be attributable to changes in the gas stored in lung from breath to breath, resulting from changes in FRC. Either nitrogen, if the subject is breathing air, or argon, which can be maintained in the anaesthetic breathing system at near its atmospheric concentration of 0.93% vol/vol, may be used. During nitrous oxide in oxygen anaesthesia, argon was supplied to the patient breathing system using a modified T-piece and a fine metering valve placed at the fresh gas flow supply to the system. The concentration was maintained manually by reference to the inspired argon concentration measured by the mass spectrometer. In practice, after induction of anaesthesia, it was found that argon flow required relatively few adjustments, being buffered by the volume of the breathing system and the FRC of the subject.

Clinical application

After Ethics Committee approval and informed consent were obtained, we tested the accuracy of the system with seven air-breathing volunteers and two groups of 10 patients receiving general anaesthesia for elective gynaecological surgery. All patients were premedicated with temazepam 10–20 mg orally 1–2 h before operation.

Anaesthesia was induced with propofol 2–2.5 mg kg⁻¹ i.v. and tracheal intubation facilitated by suxamethonium 50–100 mg i.v. Anaesthesia was maintained with 50–70% nitrous oxide in oxygen and a propofol infusion. One group of patients breathed spontaneously throughout the procedure. The other group received vecuronium 0.1 mg kg⁻¹ and controlled ventilation with a Blease–Manley ventilator.

Air breathing volunteers. The accuracy of gas exchange measurements of oxygen and carbon dioxide made by the system was compared with standard Douglas bag techniques.

Volunteers were requested to breathe through the pneumotachograph head past the mass spectrometer

probe into an Ambu resuscitation valve (fig. 2). The valve was used to displace expired gas into a Douglas bag via a ventilatory tap. After steady breathing had been established through the apparatus, the ventilatory tap was turned and 20 expirations collected in a 10-litre Douglas bag. At the same time, measurements using the breath-by-breath system were made. After thorough mixing of the contents, the mixed expiratory concentrations of carbon dioxide and oxygen in the bag were measured using the mass spectrometer. Expiratory volume was measured by emptying the bag through a previously calibrated dry gas meter which was re-zeroed before each measurement. Haldane's transformation was applied to the contents of the bag to obtain the inspired volume and the results corrected for humidity.

Three measurements were made for each volunteer: one without equilibrium gas correction, one with argon and one with nitrogen. None of the volunteers used in the experiments was a trained breather.

Spontaneously ventilating patients. After the induction of anaesthesia and intubation of the trachea, the patients breathed a nominal 70% nitrous oxide in oxygen mixture through a Mapleson A (Magill) breathing system with an inspired argon concentration maintained at 0.93 (±0.1)% vol/vol. Anaesthesia was allowed to stabilize and then measurements were made with the system. After the measurements, patients were connected immediately to an arrangement of two Douglas bags and an Ambu valve. One Douglas bag was used to collect expired gases and the second bag, which had been pre-filled with a mixture of nitrous oxide, oxygen and argon of the same inspired concentrations, was used as the source of gas. Mixed expired gas concentrations in the Douglas bag were measured by the mass spectrometer and expired volume by dry gas meter, as described previously. Oxygen, carbon dioxide and nitrous oxide exchanges were then assessed in a manner similar to that for Douglas bag measurements with spontaneously ventilating volunteers breathing room air, but without the application of Haldane's transformation. The total inspired volume was therefore assumed to be equal to the total expired volume over the Douglas bag measurement period.

Patients undergoing controlled ventilation. Expired gas was collected for 20 breaths into a Douglas bag attached to the expiratory port of the Blease–Manley ventilator. To reduce variability and to check for leaks, the ventilator was used at fixed settings previously calibrated over several breaths using a dry gas meter, to give a measurement of inspiratory volume. The stability of these calibrated tidal volumes was checked during the experiments using an electronic anemometer (Ohmeda). Expired volumes in the Douglas bag were measured using the dry gas meter and mixed expired gas concentrations were measured by mass spectrometry. An Ambu resuscitation valve was used to supplement the valve in the ventilator circuit, to prevent mixing of inspiratory and expiratory gases at the end of the Y-piece. Inspiratory concentrations were adjusted manually by manipulating the flowmeter block on

the anaesthetic machine in response to the mass spectrometer readings. The system and Douglas bag measurements were made synchronously.

RESULTS

The results from the experiments have been processed as illustrated by figures 3 and 4, which show the results from tests on carbon dioxide exchange without correction for lung gas stores under controlled ventilation. The results have then been summarized in tables I–III for each of the groups of subjects studied.

The mean exchange measured by the system was plotted against the corresponding Douglas bag measurements and regression analysis performed. Within-patient variation per breath was considered as SD error bars (fig. 3). In subsequent tables, the results of the regression analysis have been summarized in terms of slope *m* and intercept *c*, together with their SE.

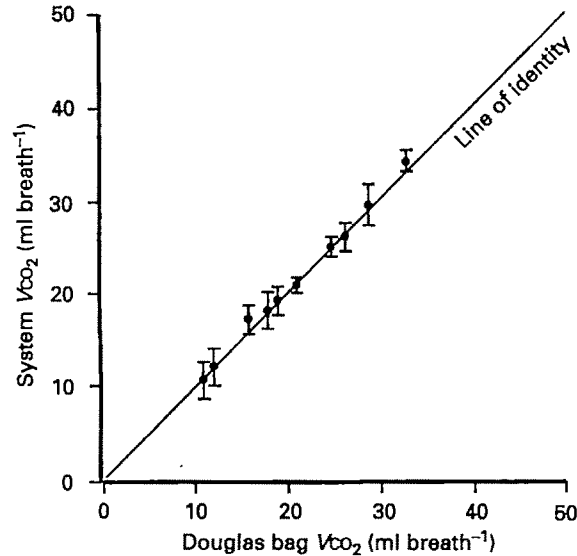


FIG. 3. Mean carbon dioxide exchange per breath: measurement by the system compared with that by Douglas bag for anaesthetized patients under controlled ventilation (no correction for lung gas) (error bars indicate ± 1 SD of within-patient, between-breath variation).

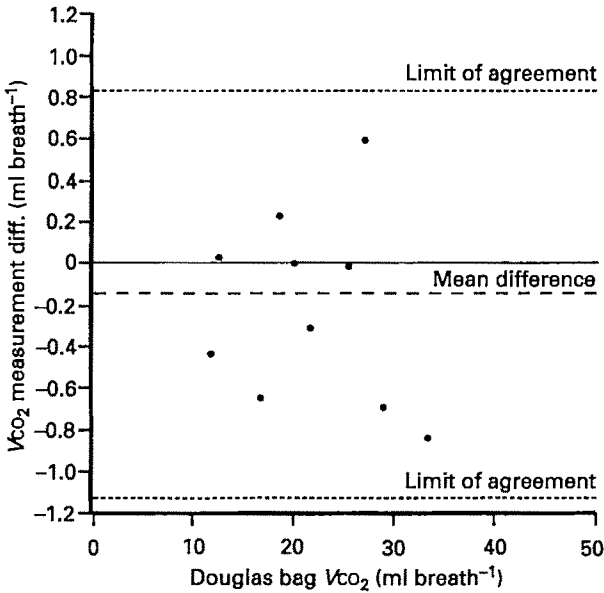


FIG. 4. Difference (system – Douglas bag) in mean carbon dioxide exchange per breath, *vs* exchange per breath measured by Douglas bag under controlled ventilation for anaesthetized patients (no correction for lung gas).

Between-subject variation (mean and SD of the differences between system and Douglas bag measurements) is given in terms of both ml breath⁻¹ and % of the Douglas bag measurement (“between-subjects differences” in tables). The within-subject, between-breath variation (SD of the differences between the breath-by-breath system measurements and the corresponding single Douglas bag measurement) is also given in terms of ml breath⁻¹ and % of the Douglas bag measurement (“within-subject, between-breath variation” in tables).

The differences between the system measurement and the Douglas bag measurement were plotted against the Douglas bag measurement (fig. 4). The limits of agreement for the measurements were calculated using the method of Bland and Altman [17] (mean error ± 2 SD). These limits are shown in figure 4 and summarized in the individual tables. In a slight departure from the method of Bland and Altman, the limits have been expressed with ref-

TABLE I. Summary of results for air breathing volunteers. S = system; DB = Douglas bag

	$V\dot{CO}_2$			$V\dot{O}_2$		
	Correction gas			Correction gas		
	None	N ₂	A	None	N ₂	A
Regression line (regression equation $(V_{\dot{x}})_S = m (V_{\dot{x}})_{DB} + c$)						
<i>m</i> (SE)	0.97 (0.03)	1.00 (0.05)	0.97 (0.06)	0.99 (0.07)	0.99 (0.09)	0.90 (0.09)
<i>c</i> (SE)	1.12 (0.65)	0.17 (1.21)	0.02 (1.35)	0.13 (2.19)	-0.18 (3.28)	-0.05 (2.98)
Between-subject differences (S – DB)						
Volume (ml) (mean (SD))	0.40 (2.06)	0.21 (2.86)	-0.56 (3.08)	0.06 (7.12)	-0.23 (8.61)	-3.26 (6.60)
Limits of agreement	-3.72/4.52	-5.51/5.93	-6.72/5.6	-14.18/14.3	-17.45/16.99	-16.46/9.94
Percent of DB (mean (SD))	4.07 (15.36)	2.32 (18.13)	-3.23 (12.57)	1.21 (26.85)	0.01 (22.90)	-11.56 (24.40)
Within-subject, between-breath variation						
Volume (SD) (ml)	4.15	5.06	5.42	12.19	8.67	10.47

TABLE II. Summary of results for spontaneously breathing patients undergoing nitrous oxide in oxygen anaesthesia. S = System; DB = Douglas bag

	V_{CO_2}		V_{O_2}		V_{N_2O}	
	Correction gas		Correction gas		Correction gas	
	None	A	None	A	None	A
Regression line (regression equation $(V_x)_S = m (V_x)_{DB} + c$)						
m (SE)	0.99 (0.02)	1.01 (0.03)	1.00 (0.03)	1.01 (0.01)	0.83 (0.09)	1.09 (0.02)
c (SE)	0.88 (0.36)	-0.77 (0.13)	0.07 (0.70)	-0.56 (0.09)	4.55 (2.41)	2.18 (0.054)
Between-subject differences (S-DB)						
Volume (ml) (mean (SD))	-0.61 (1.20)	0.60 (0.41)	-0.13 (1.82)	0.37 (0.99)	-0.11 (5.12)	-0.47 (2.20)
Limits of agreement	3.01/1.79	-0.22/1.42	-3.77/3.51	-1.61/2.35	10.35/10.13	4.87/3.93
Percent of DB (mean (SD))	-3.45 (7.39)	3.99 (3.24)	-0.50 (7.10)	-1.87 (3.59)	-0.80 (20.32)	0.28 (10.56)
Within-subject, between-breath variation						
Volume (SD) (ml)	2.0	0.70	4.45	3.59	8.05	5.35

TABLE III. Summary of results for patients undergoing nitrous oxide in oxygen anaesthesia under controlled ventilation. S = System; DB = Douglas bag

	V_{CO_2}		V_{O_2}		V_{N_2O}	
	Correction gas		Correction gas		Correction gas	
	None	A	None	A	None	A
Regression line (regression equation $(V_x)_S = m (V_x)_{DB} + c$)						
m (SE)	1.03 (0.01)	0.96 (0.01)	0.98 (0.02)	1.04 (0.01)	0.93 (0.04)	0.77 (0.06)
c (SE)	-0.57 (0.15)	0.43 (0.11)	0.53 (0.52)	-1.44 (0.18)	3.86 (1.50)	7.03 (1.87)
Between-subject differences (S-DB)						
Volume (ml) (mean (SD))	-0.15 (0.49)	0.19 (0.49)	-0.04 (1.42)	0.65 (0.94)	-1.54 (5.55)	0.26 (2.33)
Limits of agreement	-1.13/0.83	-0.79/1.17	-2.8/2.72	-1.23/2.53	-12.64/9.56	-4.4/4.92
Percent of DB (mean (SD))	-0.39 (2.33)	0.65 (3.38)	-0.60 (5.67)	4.21 (6.35)	-10.9 (34.0)	0.48 (7.62)
Within-subject, between-breath variation						
Volume (SD) (ml)	1.38	0.97	3.68	1.44	5.66	6.62

erence to the Douglas bag measurements and not the mean of the system measurements and Douglas bag measurement. Although the measurements are not exactly comparable, Douglas bag measurements have been used by other authors as a standard of comparison.

Detailed results for air-breathing volunteers are given in table I, for the exchange of carbon dioxide and oxygen according to the three methods of correction. Regression analysis showed that they were all consistent with the line of identity, with slopes m not significantly different from unity and intercepts c not significantly different from zero. For both between-subject and within-subject variation, SD values were greater for oxygen exchange than for carbon dioxide exchange; nitrogen and argon corrections did not produce any significant reduction in SD value for within-subject, between-breath variation.

In the spontaneously breathing patients, most of the regression lines were consistent with the line of identity (table II). In this group of patients, the argon correction roughly halved the between-subject SD values and reduced those between breaths. Both types of SD were again greater for oxygen than for carbon dioxide, and greater still for nitrous oxide.

For the patients receiving controlled ventilation (table III), most of the regression lines differed significantly from the line of identity, but only for nitrous oxide exchange with the argon correction was the slope notably different from unity (0.77). Again, the between-subject and within-subject SD values were greater for oxygen exchange and greatest for nitrous oxide although, here, the argon correction was not so consistently successful in reducing SD values.

DISCUSSION

General considerations of accuracy

The Douglas bag collection system is not an ideal method for strict comparison with the sort of computer-aided, breath-by-breath measurement system described: the Douglas bag collects the total gas expired over a number of breaths, but because the system measures volume per breath, a comparable mean exchange per breath can be deduced only by weighting the results by tidal volume. However, the computer system reported here did not take account of variations in tidal volume. Bearing this in mind, it is important to ascertain if the differences demonstrated between the methods in

TABLE IV. Comparison of breath-to-breath coefficients of variation in results of the system reported here and other systems described in the literature for air breathing subjects (mean (SD between subjects)). *Trained or regular breather; **patients, untrained or irregular breathers

Investigating group and method	Coefficient of variation	
	CO ₂	O ₂
Swanson [17]		
No correction gas		21.6 (6.3)
Nitrogen correction		10.2 (3.2)
Other correction		5.3 (1.6)
Goldman [8]		
No correction gas	13.1*	7.3
	46.2**	56.2
Nitrogen correction	21.4*	26.8
	27.4**	40.7
	13.9*	8.3
Other correction	22.3**	20.9
	13.1*	8.2
	22.1**	21.0
Wessel [9]		
Nitrogen correction	17.54 (5.96)**	16.59 (5.90)
Beaver [10]		
Work rate: Rest	9.63 (2.21)	11.11 (1.34)
50 W	7.06 (1.26)	8.23 (2.78)
100 W	6.33 (2.91)	5.26 (3.10)
Mean of results from present system		
No correction gas	15.21 (6.6)	23.50 (6.2)
Nitrogen correction	18.20 (8.7)	20.69 (12.2)
Argon correction	20.60 (14.2)	20.42 (10.6)

this study indicate a limit of accuracy less than that seen in dual flow transducer systems used by others [8–10, 17] which used different methods of accounting for the fundamental difficulties of making this type of measurement.

In all types of subject and for all types of correction, the errors associated with carbon dioxide measurement were smaller than those associated with oxygen or nitrous oxide. This was to be expected, and was caused by the errors of inspiratory volume measurement being cancelled by the negligible inspiratory concentrations of carbon dioxide present in the breathing systems used. If a breath-by-breath measurement technique of the type reported was used to assess gas exchanged at increased inspiratory concentrations of carbon dioxide, it would be expected that the overall errors for carbon dioxide measurement would increase and become comparable to those of oxygen measurement.

In some cases, the limits of agreement for the methods indicated large volume per breath differences between different corrections and subject groups. In the case of air-breathing volunteers, these differences may be as much as $\pm 50\%$ —an unacceptable figure. However, bearing in mind the limited comparability of Douglas bag measurements and the system measurements, conclusions drawn from the limits of agreement have to be treated with a degree of caution.

Comparison of system with other systems for air breathing volunteers

Table IV shows the coefficients of variation (CV) for gas exchange measurements taken from the literature for measurements in air-breathing sub-

jects. The CV expresses variability within single-subject records. The use of CV as an indicator of measurement reliability had the advantage that the way in which results were originally expressed made no difference—a necessary property if different systems from the literature, that vary greatly in their method of reporting, are to be compared.

The disadvantage of CV is that it does not take account of any effects of error of distribution in the data that varies from system to system. However, CV does give an approximate basis of comparison.

Where more than one subject data set was available, the mean and SD of the individual CV for published subject records are shown. There was no significant difference between the variability of the results reported here and those of Swanson, Sodal and Reeves for no lung gas stores correction [16] or Wessel and colleagues [9] for the same methods implemented with a dual transducer system. The more revealing of these two studies is that of Wessel's group, as they made measurements in subjects at rest. Swanson, Sodal and Reeves [16] used what appear to have been trained breathers under moderate exercise.

There are significant differences between our results and those of Goldman, Dzvonczyke and Yadagani [8] and Beaver, Lamaura and Wasserman [10]. Beaver's group [10] used trained breathers and the experiments were performed at steadily increasing exercise workloads. It might be thought that, as exercise levels increased, the amount of between-breath variation would increase. However, their results [10] suggest the reverse because, as the workload increased, CV values decreased. Goldman's group [8] classified their results according to subjects being regular or irregular breathers. Our results were generally significantly less than those from the irregular breathers group and significantly greater than those from the regular breathers group. Both groups were at rest and were volunteers although, in the case of the regular breathers group, they were likely to have been trained breathers.

It seems that, in common with other breath-by-breath measurement systems, the system reported here was sensitive to variation in ventilatory pattern. Its degree of sensitivity is comparable to that of other systems using the same measurement methods and dual pneumotachographs in the absence of anaesthetic gases. The results were consistent with the present system being as reliable as any other and superior in some cases, even with respect to irregular breathing by untrained volunteers.

Adequacy of accuracy for clinical measurements during general anaesthesia

One of the potential advantages of using breath-by-breath measurements during anaesthesia is that it is a universal method of measurement of gas exchange requiring little interference in the patient breathing system.

Alternative methods of measurement of gas exchange during anaesthesia have typical estimated accuracies for oxygen and carbon dioxide exchange measurements of 3.0% (SD) and for nitrous oxide uptake of 7.0% (SD) [1, 5, 7]. With these systems,

significant clinical differences have been shown in gas exchange and their accuracies are comparable to that of the system reported here for spontaneous and controlled ventilation during general anaesthesia.

Efficacy of lung gas stores correction

The effects of the applications of correction for variation in lung gas stores by nitrogen or argon were ambiguous. In the case of air-breathing volunteers, neither of the two methods of correction for gas lung stores offered definitively greater accuracy, probably because of limitations of accuracy inherent in the method in the presence of irregular breathing patterns. Even in this case, however, argon correction was no worse than the nitrogen correction, even though the inspired concentration was two orders of magnitude smaller. It would seem that argon correction is a viable alternative to nitrogen correction.

In the case of the application of the correction during general anaesthesia, significant improvement for spontaneous ventilation was seen in all cases, but only a marginal increase in accuracy was achieved by the application of the corrections for controlled ventilation. This was to be expected as, under controlled ventilation, changes in lung gas stores and breath-by-breath would be expected to be smaller.

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TIME COURSE OF PRIMARY AND SECONDARY HYPERALGESIA AFTER HEAT INJURY TO THE SKIN

S. MØINICHE, J. B. DAHL AND H. KEHLET

SUMMARY

We have examined the time course of, and relationship between, primary and secondary hyperalgesia after thermal injury to the skin in humans. Burn injuries (15 × 25 mm rectangular thermode, 49 °C, 5 min) were produced in eight healthy, unmedicated male volunteers, on the medial side of the right calf, on two occasions at least 8 days apart. Heat pain detection thresholds (HPDT), heat pain tolerance (HPT), mechanical pain detection threshold (MPDT) and the intensity of burn-injury induced erythema (skin erythema index, SEI) were assessed inside the burn injury. HPT was assessed only in one study period. Areas of hyperalgesia to pinprick and brush were determined outside the injury. Assessments were made before and regularly for 72 h after the burn injury. There was a decrease in heat pain and mechanical thresholds, an increase in SEI inside the injury and development of mechanical hyperalgesia outside the injury. These alterations were consistent between the two examination days. The time course of the intensity of primary hyperalgesia was related closely to that of changes in area of secondary hyperalgesia, and hyperalgesia outside the injury did not outlast hyperalgesia inside the injury in any volunteer. These findings suggest post-injury development of secondary hyperalgesia to be a dynamic process, closely related in time to a peripheral nociceptive input, with reversal to normal when the peripheral lesion disappears. These observations may be relevant to the concept of "pre-emptive" analgesia. (Br. J. Anaesth. 1993; 71: 201–205)

KEY WORDS

Analgesia: pre-emptive. Pain: experimental, mechanism.

Cutaneous injury leads to alterations in thermal and mechanical sensibility termed primary and secondary hyperalgesia [1–4]. "Primary hyperalgesia" refers to changes within the injury and "secondary hyperalgesia" to changes in the undamaged tissue surrounding the injury. Primary hyperalgesia is probably caused by sensitization of both peripheral nociceptors and central neurones [4–10]. A recent series of studies in humans and primates supported the previous suggestion [2, 3] that secondary hyperalgesia is caused by a central facilitation of mechanoreceptive input from the periphery [4, 6–9].

Corresponding experimental studies have demonstrated that central sensitization of dorsal

horn neurones may outlast the peripheral injury [11] and that it may be eliminated or reduced by pre-, but not post-injury neural block or administration of opioids [12–15]. These findings suggest that the central alterations, when induced, may be independent of a sustained nociceptive input from the periphery. This dissociation between the alterations in the peripheral and in the central nervous system is critical for the clinical implications of "pre-emptive analgesia" [16–19]. However, the relationship between peripheral and central alterations after prolonged peripheral activation of nociceptive processes has not been evaluated systematically in clinical studies.

The aim of the present study was to examine the time course of, and relationship between, primary and secondary hyperalgesia after thermal injury to the skin in humans.

SUBJECTS AND METHODS

We studied eight healthy, unmedicated male volunteers, median age 28 yr (range 20–46 yr). Informed consent was obtained from all volunteers, and the study was approved by the regional Ethics Committee.

Hyperalgesia was produced by burn injuries at analogous skin areas on the medial surface of the right calf with a 15 × 25 mm rectangular thermode (Thermotest, Somedic A/B, Sweden), applied to the skin with a standardized pressure (26 kPa) [20]. The temperature of the thermode was 49 °C, and the application time 5 min. This resulted in first- or second-degree burn injuries with blisters measuring approximately 0.25–0.5 cm². During thermal stimulation, intense pain was experienced for the first 1–3 min, and then declined gradually towards a feeling of soreness through the rest of the conditioning period.

Primary hyperalgesia

Thermal thresholds inside the injury were determined with a computerized Thermotest (Somedic AB, Sweden). The thermode was identical with that used for production of the burn injury. Heat pain detection threshold (HPDT) was defined as the least temperature perceived as painful and heat pain

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tolerance (HPT) as the maximal tolerable temperature. Interpretation of "pain" was left to the subject, who was instructed to apply the same interpretation throughout the study. Determinations were made from a baseline temperature of 32 °C, a temperature gradient of 1 °C s⁻¹ and a maximum temperature of 52 °C. The subjects were instructed to activate a push button when a sensation of pain was perceived (HPDT) or when maximal tolerable pain was perceived (HPT). Each threshold was calculated as the average of three determinations performed with intervals of 10 s between each stimulation. Pain thresholds to *mechanical stimulation* (mechanical pain detection threshold, MPDT) were determined by pinprick with nine successive rigid nylon Von Frey hairs (1 = 0.5 g; 9 = 16 g) (Somedic A/B, Stockholm, Sweden). MPDT was defined as the smallest pressure (pinprick) in the burn injury which produced a sensation of pain.

As an estimate of the severity of inflammation, the intensity of burn injury-induced *erythema* inside the burn injury was assessed with a hand-held skin reflectance spectrophotometer (Dermaspectrometer, Cortex Technology, Hadsund, Denmark) [21, 22] applied to the centre of the test areas. The spectrophotometer provided a skin erythema index (SEI) based on the absorption characteristics of green and red light at 568 nm and 655 nm in the skin.

Secondary hyperalgesia

The area of mechanical hyperalgesia that developed around the burn injury was assessed with a rigid (16-g) Von Frey hair (pinprick), and a hand-held brush with multiple nylon filaments covering an area of 6 × 12 mm (brush). Borders of hyperalgesia were determined by stimulating along eight radial, linear paths arranged from the centre of injury. Stimulation along each path began outside the hyperalgesic area, where no sensation of pain was perceived, continuing towards the centre of the burn injury until the subject reported a pain level of 6 on a

TABLE I. Heat pain detection thresholds (HPDT), heat pain thresholds (HPT), mechanical pain detection thresholds (MPDT), and erythema inside the injury ("primary hyperalgesia"), and "secondary hyperalgesia" to pinprick and brush outside the injury, before ("pre") and 3–72 h after burn injury on the two examination days (median (range)). *Significantly different from baseline (pre) values (P < 0.05, with Bonferroni's correction)

	Day 1	Day 2
Heat pain detection threshold (HPDT)		
Pre	46.6 (44.2–49.4)	46.3 (44.4–49.2)
3 h	41.1 (38.5–45.7)*	42.0 (38.4–45.9)*
6 h	42.2 (38.3–45.9)*	45.4 (39.8–47.7)
24 h	43.4 (40.3–46.0)*	44.3 (41.2–45.4)*
30 h	43.4 (40.6–46.0)*	44.2 (42.8–46.3)
48 h	44.6 (40.6–46.5)*	43.8 (42.1–48.7)
54 h	44.9 (40.0–46.9)	45.2 (42.1–47.7)
72 h	45.1 (41.8–48.9)	46.1 (42.1–47.7)
Heat pain threshold (HPT)		
Pre		50.7 (48.9–52.0)
3 h		49.0 (45.1–50.8)*
6 h		49.4 (45.0–51.1)
24 h		48.6 (46.6–51.0)*
30 h		49.6 (47.3–51.1)
48 h		49.8 (49.1–52.0)
54 h		49.9 (49.1–52.0)
72 h		50.1 (48.5–52.0)
Mechanical pain detection threshold (MPDT)		
Pre	9 (9–9)	9 (9–9)
3 h	5 (4–8)*	6 (5–7)*
6 h	6.5 (5–9)*	5.5 (5–7)*
24 h	7 (6–9)	6.5 (4–8)*
30 h	6.5 (6–9)*	6 (5–8)*
48 h	8 (6–9)	9 (7–9)
54 h	8.5 (7–9)	9 (6–9)
72 h	9 (7–9)	9 (9–9)
Erythema		
Pre	13.5 (7–16)	13.5 (7–19)
3 h	20 (18–23)*	20.5 (17–25)*
6 h	19.5 (17–23)*	21.5 (16–25)*
24 h	20 (19–24)*	21 (14–28)*
30 h	22 (19–24)*	21.5 (15–25)*
48 h	20 (15–24)*	23 (10–24)*
54 h	18.5 (12–21)	20.5 (10–24)
72 h	17 (10–22)	17 (10–23)
Secondary hyperalgesia, pinprick		
Pre	0 (0–0)	0 (0–0)

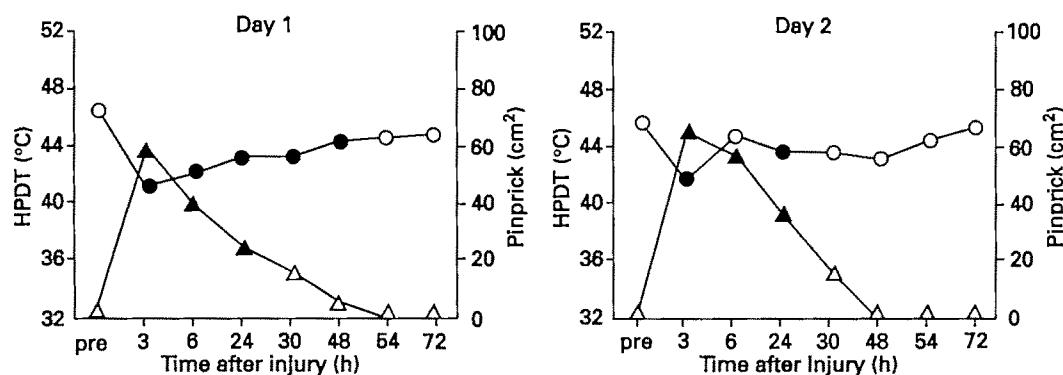


FIG. 1. Relationship between heat pain detection thresholds (HPDT) (the least temperature perceived as painful) inside the injury (●) and area of hyperalgesia to pinprick outside the injury (Pinprick) (▲) on two examination days after thermal injury (median values; $n = 8$). Open symbols: baseline (pre) values and values not significantly different from baseline. Closed symbols: values significantly different from baseline ($P < 0.05$ with Bonferroni's correction).

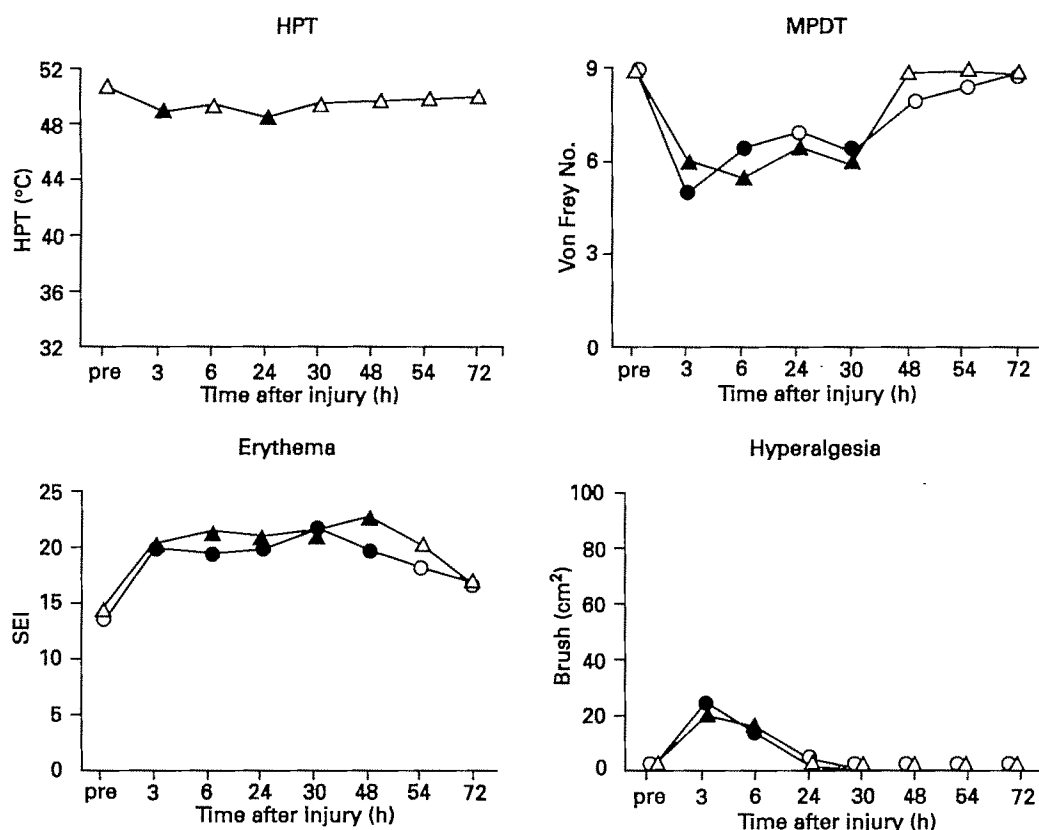


FIG. 2. Effect of thermal injury on changes in heat pain threshold (HPT) (the maximal tolerable temperature) inside the injury; mechanical pain detection threshold (MPDT) (the least pressure perceived as painful (Von Frey No.)) inside the injury; skin erythema index (SEI) (intensity of burn injury-induced erythema) inside the injury; area of hyperalgesia to brush outside the injury (Brush) on two study days (1 = ●; 2 = ▲) > 1 week apart (median values, $n = 8$). Open symbols: baseline (pre) values and values not significantly different from baseline. Closed symbols: values significantly different from baseline ($P < 0.05$ with Bonferroni's correction).

Primary hyperalgesia

HPDT (fig. 1A) decreased significantly (in eight of eight volunteers) between 3 h and 48 h after injury, compared with preinjury thresholds ($P < 0.0001$ (F)). Results were similar on day 2 ($P < 0.0008$ (F)), except that HPDT was decreased in only seven of the eight volunteers at 6, 30 and 48 h after injury, explaining the non-significant difference from pre-injury thresholds at these assessments (fig. 1B). HPT (fig. 2A) decreased after injury ($P = 0.01$ (F))

and remained decreased until 24 h after injury compared with preinjury threshold. MPDT (fig. 2B) decreased significantly between 3 and 30 h after injury on both examination days, compared with preinjury thresholds ($P < 0.00001$ (F)). HPDT and MPDT were not significantly different on the two examination days ($P = 0.38$ (NP) and $P = 0.74$ (NP), respectively).

SEI (fig. 2C) increased between 3 and 48 h after injury on both examination days, compared with preinjury values ($P < 0.00001$ (F)). SEI was not

significantly different on the two examination days ($P = 0.74$ (NP)). An inverse relationship was observed between SEI and HPDT on both examination days (day 1: $R_s = -0.57$, $P < 0.00001$; day 2: $R_s = -0.44$, $P = 0.0004$; global data).

Secondary hyperalgesia

Areas of hyperalgesia to pinprick (fig. 1A, B) and brush (fig. 2D) increased significantly between 3 and 24 h, and between 3 and 6 h after injury, respectively ($P < 0.00001$ (F) for both pinprick and brush, compared with pre-injury areas (= zero)). Areas of hyperalgesia to pinprick and brush were not significantly different between the two examination days ($P = 0.55$ (NP) and $P = 0.64$ (NP), respectively).

Relationship between primary and secondary hyperalgesia

An inverse relationship was observed between HPDT inside the injury and the area of hyperalgesia to pinprick and brush outside the injury on both examination days (day 1: pinprick $R_s = -0.58$, $P < 0.00001$; brush $R_s = -0.42$, $P = 0.0009$; day 2: pinprick $R_s = -0.39$, $P = 0.002$; brush $R_s = -0.32$, $P = 0.01$, global data). The relationship between HPDT and areas of secondary hyperalgesia to pinprick is shown in figure 1 (A, B). Small areas of secondary hyperalgesia to pinprick were still present in seven, six and two volunteers at 30, 48 and 54 h, but in no subject 72 h after the injury. Likewise, small areas of hyperalgesia to brush were present in five, three, two and one subjects at 24, 30, 48 and 54 h, but in none 72 h after injury. When HPDT inside the injury had returned to normal, areas of hyperalgesia to pinprick or brush outside the injury could not be demonstrated in any subject.

A significant correlation was observed between HPDT and MPDT inside the injury (day 1: $R_s = 0.34$, $P = 0.007$; day 2: $R_s = 0.41$, $P = 0.001$, global data).

DISCUSSION

In agreement with previous studies [1–4], we observed a decrease in heat pain and mechanical thresholds inside, and development of mechanical hyperalgesia outside, a thermal injury to the skin. In addition, an increase in SEI was observed inside the injury.

The molecular mechanisms of primary hyperalgesia to heat stimuli are probably related closely to the processes of inflammation [5, 10]. Following noxious stimuli to peripheral nerves, antidromic impulses in axons promote the release of substance P from nerve endings, resulting in vasodilatation and increase in vascular permeability. This leads to local oedema and flare and, with the release of substances such as prostaglandins, leukotrienes, bradykinin, serotonin and histamine, to inflammation and sensitization of nociceptors [5, 10].

We observed an inverse relationship between SEI and HPDT on both examination days. The skin erythema index may be useful in evaluating the dynamics of inflammation [23], but further studies are needed to evaluate the relationship between SEI and nociceptive processes.

From clinical studies there is substantial evidence that secondary hyperalgesia resulting from a physical injury [2, 3] or application of capsaicin to the skin [4, 6–9] is caused by central facilitation of mechanoreceptive input from the periphery. It is not clear if the central facilitation is independent of a continuous input to the spinal cord and, thus, may outlast the duration of the peripheral injury. Hardy, Wolff and Goodell [2] reported that tenderness at the site of a thermal injury to the skin always outlasted secondary hyperalgesia. However, exact data on the time course of primary and secondary hyperalgesia were not provided in that study. In contrast, hyperalgesia for punctate stimuli outside an intradermal injection of capsaicin lasted 13–24 h, whereas flare and hyperalgesia to heat near the injection site lasted only for 1–2 h [4]. Post-injury administration of local anaesthetics at the site of a thermal injury [1, 20] or after an intradermal injection of capsaicin [4] only partially reduced the area of secondary hyperalgesia during short-term (< 3 h) studies. These findings may reflect a period of at least a few hours during which there is relative independence of central sensitization to ongoing peripheral activity. In contrast, the abnormal central state in sympathetically maintained pain depends on a continuous input to the central neurones, as a block of the nociceptor input normalizes the central functional changes [10, 24].

In experimental studies some, but not all, central alterations may be dependent on peripheral activity [25]. Thus Dickenson and Sullivan [26] showed that central changes produced by formalin hyperalgesia could be reversed by afferent block, whereas other studies have found that post-injury administration of local anaesthetics at the site of injury produces only partial and slow reversal of expansion in receptive fields and hyperexcitability [12, 13, 27, 28]. However, those studies were also short-term observations within less than 1–2 h after afferent block.

We observed a significant relationship between primary and secondary changes, but hyperalgesia outside did not outlast hyperalgesia inside the injury in any volunteer. It has been suggested that hyperalgesia to mechanical stimuli inside the injury may be caused by central neuronal changes [7, 10]. In our study, the decrease in MPDT correlated with, but did not outlast, the decrease in HPDT inside the injury. Our findings, which were consistent between two examinations, are in agreement with the original observations by Hardy, Wolff and Goodell [2] and may suggest that maintenance of the central alterations after thermal injury is dependent upon a sustained barrage of afferent impulses [2].

Based on experimental studies, it has been speculated that surgery in humans may lead to alterations in central neuronal processing, and that such alterations may outlast the peripheral injury and amplify and prolong postoperative pain [11, 16, 17]. Although the time-course of primary and secondary hyperalgesia after a thermal injury may differ from that of surgery, results from the present clinical study cannot confirm the suggestion that central sensitization may outlast afferent transmission from a peripheral injury [27]. Therefore, the

relative contribution of secondary hyperalgesia to the magnitude and duration of postoperative pain awaits clarification.

Finally, our observations may be relevant to the concept of "pre-emptive" analgesia, as analgesic treatment which continues as long as the afferent barrage from the periphery (surgical wound) continues may be more important than a short-lasting pre-emptive treatment. Therefore, pre-emptive analgesia should be provided in combination with prolonged treatment. It is not clear if pre-emptive analgesia may facilitate or shorten the postoperative need for analgesics [18].

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TEACHING FIBROPTIC NASOTRACHEAL INTUBATION WITH AND WITHOUT CLOSED CIRCUIT TELEVISION

J. E. SMITH, S. G. FENNER AND M. J. KING

SUMMARY

We have compared the progress of anaesthetists taught fiberoptic nasotracheal intubation with the aid of a closed circuit television (CCTV) system with that of anaesthetists taught by traditional methods. Twenty anaesthetists were allocated randomly to either the video or traditional training group. A graduated training programme was used in which the first stage was an introduction to techniques and apparatus and the second stage was practice on an airway model. During the third stage, rhinoscopy, pharyngoscopy and laryngoscopy were performed on anaesthetized patients whose lungs were ventilated via an orotracheal tube. A maximum time of 6 min was allowed for completion of laryngoscopy. Trainees aimed to perform five endoscopies in less than 60 s before moving on to the next stage. During the fourth stage, they carried out five traditional nasotracheal intubations (plus two video-controlled intubations for the video group) on apnoeic, anaesthetized patients. The mean number of endoscopies required (11.7 vs 21.8), mean total endoscopy time (21.5 vs 63.1 min) and mean number of failed endoscopies (0.8 vs 3.9) were significantly less in the video group. All the video-controlled intubations were successful. There was no significant difference between the number of successful traditional intubations in the two groups (90% video, 92% traditional). CCTV appears to enhance substantially the rate of acquisition of fiberoptic nasotracheal intubation skills. (Br. J. Anaesth. 1993; 71: 206–211)

KEY WORDS

Intubation: technique, training. Equipment: fiberoptic laryngoscope.

Fiberoptic intubation is an important advance in the management of the difficult airway and there is a growing consensus that opportunities to learn this technique should be on the curriculum of every anaesthetist in training [1–3]. Fiberoptic airway endoscopy demands acquisition of complex psychomotor skills and is sometimes perceived as a difficult technique to learn. Experienced endoscopists should therefore attempt to evaluate techniques and apparatus that may facilitate and enhance the learning of fiberoptic skills.

Training may begin directly with anaesthetized patients, with or without the use of special pharyn-

geal airways and guides [4–7]. However, there are likely to be advantages in adopting a step-by-step approach of trainees graduating in stages from simple to more complex tasks [8, 9]. Hence, training may begin with practice on an airway model, followed by laryngoscopy in anaesthetized patients and, subsequently, fiberoptic intubation in awake patients. Graduated training programmes or progressive part practice are recognized as efficient ways to learn complex motor skills [10].

A major obstacle to progress in traditional teaching is that the trainee is the only eyewitness to the attempted endoscopy. Effective tuition in these circumstances is difficult and the trainee learns largely by a process of trial and error. The miniature video camera enables the optical image from the fibrescope to be displayed on a television screen, so that both instructor and trainee can view the endoscopy. The instructor should therefore be able to make a more substantial teaching contribution [11]. This study was designed to assess to what extent, if any, closed circuit television (CCTV) systems could enhance the rate of acquisition of fiberoptic skills, by comparing the progress of two groups of anaesthetists, one receiving training with the aid of video display and the other with traditional teaching methods.

PATIENTS AND METHODS

The study was approved by the Ethics Committee of the South Birmingham Health District. Three experienced endoscopists taught the practical skills of fiberoptic intubation to 20 anaesthetists who had no previous experience in fiberoptic techniques. Trainees were paired according to age and professional status then assigned on a random number basis to receive teaching either by conventional methods (traditional group) or with the aid of CCTV (video group). The training schedule was divided into four stages:

Stage 1—Introduction

Instructors demonstrated operation of the Olympus LF1 fiberoptic laryngoscope and ancillary

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equipment to all trainees. They also familiarized trainees in the video group with the CCTV system, which comprised the Olympus OTV-S2 endoscopic camera and control unit, video cassette recorder and television monitor. All trainees received oral and written information on the main problems likely to be encountered during fibreoptic intubation and the methods used to solve them.

Stage 2—Practice on bronchoscopy model

Trainees practised manipulating the fibroscope on a Laerdale intubation trainer with tracheobronchial tree model under the supervision of an instructor. They aimed to locate and demonstrate the carina and the main and lobar bronchi. They then advanced the fibroscope further down the airway to examine the representations of the segmental bronchi. Instructors emphasized the importance of keeping the fibroscope tip in the centre of the airway and moving it in the desired direction by using a combination of deflection control lever movements and axial rotation of the instrument. The fibroscope insertion tube was kept fully extended to prevent its bowing. This helped produce effective axial rotation; otherwise, the bow tended to neutralize this movement.

Trainees in the traditional group used the fibroscope in the conventional manner, relinquishing the eyepiece from time to time, to enable the instructor to observe and comment. Trainees in the video group practised with the endoscopic camera attached to the eyepiece so that both trainee and instructor could observe practice continuously. Instructors gave practical advice and guidance as necessary. As an additional exercise, only possible in the video group, instructors asked trainees to direct the fibroscope into specific segmental bronchi observed on the television screen. They selected those airways in which intubation was possible only after complex manipulations of the fibroscope, including rotational movements.

A minimum of 30 min was spent on the bronchoscopy model initially and trainees had the option of returning to it during subsequent stages, if necessary.

Stage 3—Endoscopies

During the third stage, trainees performed rhinoscopy, pharyngoscopy and laryngoscopy in anaesthetized, ASA group I or II patients (ages 16–70 yr) requiring mainly elective general or Ear, Nose and Throat surgery for which the trachea had been intubated. Patients were anaesthetized with glycopyrronium, fentanyl, propofol and atracurium. The airway was secured with an oral tracheal tube, usually size 8 mm for males and size 7 mm for females, using a Macintosh laryngoscope. The lungs were ventilated with nitrous oxide and isoflurane in oxygen, using a Penlon 200 automatic ventilator attached to a Bain anaesthetic system. Tidal volume and minute volume were adjusted to maintain the expired carbon dioxide partial pressure at 4.8–5.3 kPa. ECG, arterial pressure, arterial oxygen saturation and carbon dioxide concentrations were monitored. Arterial oxygen saturation was recorded

at 1-min intervals and a note was also made of the smallest value observed.

A skilled anaesthetic assistant extended the patient's cervical spine and displaced the mandible anteriorly with the mouth open. Initially, instructors demonstrated each endoscopy, with the trainees in the traditional group making intermittent observations and those in the video group observing throughout the procedure. The primary goal was to maintain a clear view of all the anatomical features of the airway during the advancement of the fibroscope, by making controlled and co-ordinated manipulations of the tip. The trainee, therefore, conducted each endoscopy in the following manner. He advanced the fibroscope tip approximately 1 cm into each nostril in turn, identified the septum, floor of the nose and inferior and middle turbinates and assessed the size of each nasal cavity. He then directed the fibroscope alongside or below the inferior turbinate of the most patent nasal passage. When the instrument reached the nasopharynx (where the axis of the airway made a right angled turn), contact with the posterior pharyngeal wall was avoided by deflecting the fibroscope tip anteriorly and making any rotational movements necessary to re-centralize the view at this point. After confirming an adequate separation between the soft palate and posterior pharyngeal wall, the trainee identified the uvula, tracheal tube and larynx, and manipulated the fibroscope anteriorly between one of the ary-epiglottic folds and the tube, thence towards the anterior aspect of the vocal cords. He passed the tip of the instrument between the vocal cords or, if this was not possible, to the level of the anterior commissure, to complete the endoscopy. The instructor contributed practical advice, constructive criticism and further demonstrations to both groups of trainees as necessary. As difficulties occurred, instructor and trainee discussed practical solutions and how the difficulties could be avoided. A debriefing session followed each endoscopy, with the aid of the video tape recording of the procedure for video group trainees.

The time taken to complete each endoscopy was recorded. Trainees aimed to complete five endoscopies, each in less than 60 s, before moving on to the next stage. Any endoscopy not completed within 6 min was deemed a failed endoscopy and abandoned.

Stage 4—Intubations

Trainees performed fibroscope-guided nasotracheal intubations on anaesthetized ASA I patients (aged 16–50 yr) undergoing elective oral surgery. Patients were non-obese, not expected to present difficult tracheal intubation and had no evidence of nasal obstruction. They were anaesthetized with glycopyrronium, fentanyl, propofol and atracurium and the lungs were ventilated manually with isoflurane in oxygen using a face mask attached to a Bain anaesthetic system. Trainees in the video group performed the first two intubations under video control. They then converted to the conventional technique, after a practice session on the mannikin to accustom themselves to the image seen

when looking directly into the fibroscope eyepiece. Both groups of trainees attempted five traditional fibreoptic nasotracheal intubations. If the trainee had not been able to direct the fibroscope into the trachea within 60 s, the attempt was abandoned and deemed a failed intubation. The lungs were re-ventilated with isoflurane in oxygen and the intubation was carried out with a Macintosh laryngoscope.

The two groups were compared using the Wilcoxon signed rank test.

TABLE I. Age, status, years in anaesthesia and sex distribution of the two study groups (mean (SEM) [range] where applicable)

Group	Age (yr)	Professional status	Time in anaesthesia (yr)	Sex (M/F)
Traditional (n = 10)	37.9 (3.2) [28–55]	4 Consultants 3 Registrars 3 SHO	11.0 (3.4) [1–31]	7/3
Video (n = 10)	36.8 (3.8) [27–63]	4 Consultants 3 Registrars 3 SHO	10.8 (3.3) [1–30]	6/4

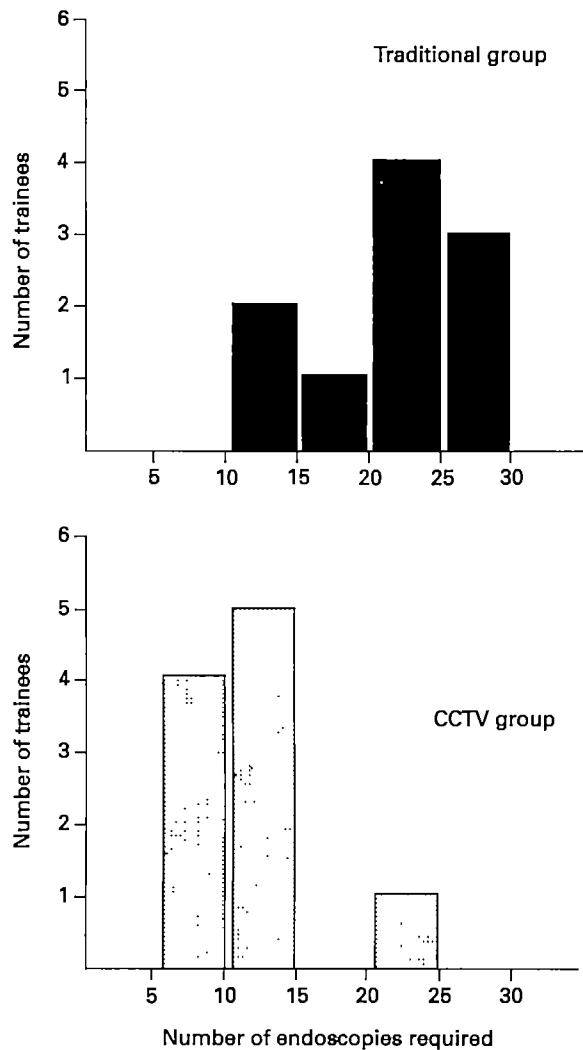


FIG. 1. Frequency histograms of number of endoscopies required during stage 3 (endoscopies), for traditional and CCTV groups.

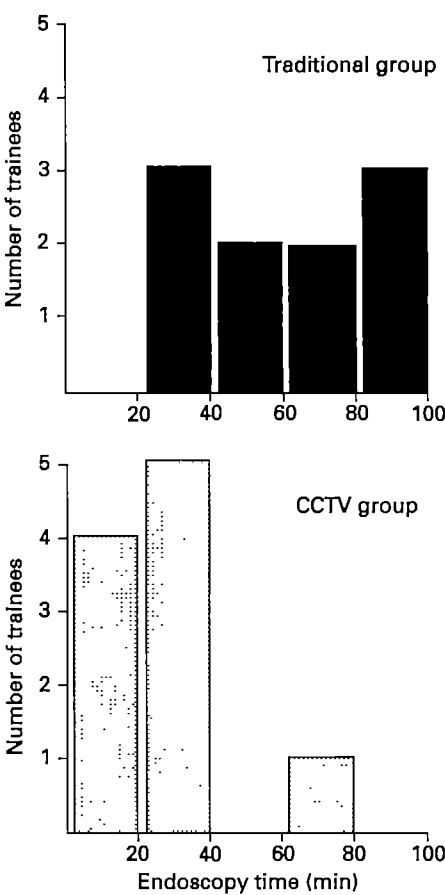


FIG. 2. Frequency histograms of total endoscopy times required during stage 3 (endoscopies) for traditional and CCTV groups.

RESULTS

The two groups were similar in age, professional status, number of years in anaesthetic practice and gender (table I).

Three hundred and thirty-five fibreoptic endoscopies and 120 fibreoptic intubations were performed by the 20 trainees. Frequency histograms of the number of endoscopies required and total endoscopy times required by the two groups during stage 3 of training are shown in figures 1 and 2. The video group required significantly fewer practice endoscopies, significantly less endoscopy time and had significantly fewer endoscopy failures than the traditional group (table II).

The video group had a 100 % success rate with the video-controlled intubations. There was no significant difference between the success rates for the traditional intubations between the video (90 %) and the traditional (92 %) group.

TABLE II. Mean (SEM) [range] number of endoscopies, total endoscopy time and number of endoscopy failures in the two study groups. *P < 0.05 compared with traditional group

Group	Number of endoscopies	Total endoscopy time (min)	Number of failed endoscopies
Traditional (n = 10)	21.8 (1.8) [13–29]	63.1 (8.1) [22.8–96.6]	3.9 (1.0) [0–10]
Video (n = 10)	11.7* (1.4) [8–23]	21.5* (5.5) [7.2–67.2]	0.8* (0.49) [0–5]

There were no significant changes in arterial oxygen saturation during the endoscopies and intubations. The smallest observed value of oxygen saturation was 96% during the endoscopies and 94% during the intubations.

DISCUSSION

Fibreoptic airway endoscopy is a complex psychomotor skill involving interactions of perception, response selection and movement control. A key feature of this type of skill is that performance level can be greatly improved by deliberate practice [10]. The aim should be to achieve smooth and controlled fibrescope movements that produce a continuous view of all the features of the upper airway, without periods of obscured vision or disorientation. Continuity of direct vision is emphasized because ultimately it leads to consistency and efficiency and because complete familiarity with the endoscopic appearance of the normal airway is a prerequisite for successful clinical assessment of a difficult or pathological airway.

Several training programmes have been introduced to help anaesthetists develop this high standard of proficiency in a systematic way [4-7]. As an alternative to the "see one, do one" approach, Ovassapian and colleagues advocated a graduated training programme [8, 12]. Initial practice on a bronchoscopy teaching model was followed by laryngoscopies on patients recovering from anaesthesia, which in turn was followed by nasotracheal intubation in awake, sedated patients under topical anaesthesia. The concept that skills acquired with relatively simple models or simulators can, to some extent, be successfully transferred to the real-life situation is an important principle in education [13]. The more realistic the simulator is, the more valuable is the experience gained. Hence, the respiratory tracts of the cadaver and the anaesthetized pig [14] have been evaluated and recommended as training models, as both appear to resemble closely the conditions encountered in clinical practice.

After initial practice on an airway simulator, the next stage is practice on patients under local or general anaesthesia, or both. During the third part of Ovassapian's programme, trainees had the opportunity to perform fibreoptic nasotracheal intubations in awake patients presenting for elective oral surgery. This technique undoubtedly provides good practice conditions but, at present, the number of centres in which awake intubations are performed routinely is limited. As an alternative, practice in ENT outpatients [15] or in patients undergoing diagnostic bronchoscopy under local anaesthesia [3, 16] has recently been proposed.

General anaesthesia may also provide satisfactory conditions for fibreoptic training. Endoscopies can be performed on apnoeic patients, on patients breathing spontaneously or on patients receiving mechanical ventilation of the lungs after tracheal intubation. When trainees practise with apnoeic patients, it is mandatory that hypoxia does not occur. Ideally, early endoscopy practice should be un-

hurried and unpressurized if accurate and enduring motor skills are to be assimilated. However, in one study of orotracheal fibreoptic intubation [17], four resident anaesthetists were successfully trained with hyperventilated, preoxygenated, apnoeic patients. A total of 91 intubations was performed and, although there was a decrease in oxygen saturation in some patients, it was never to less than 95%. The use of a teaching adapter probably made a significant contribution to the achievements of the trainees in this trial. There is no information on whether such satisfactory results would be possible without this aid.

Unfortunately, in a wider review of this method of teaching [18], it was apparent that a small number of obese patients rapidly become desaturated and needed emergency reoxygenation. Because of anxiety about this attendant risk in apnoeic patients, an anaesthetic technique involving spontaneous ventilation has been suggested [7]. It uses an oropharyngeal Guedel airway with a Nosworthy chimney attached to an anaesthetic breathing system that assists in airway maintenance and allows ventilation to be controlled if necessary. Trainees are then free to perform nasotracheal endoscopy followed by fibrescope-guided intubation (after the administration of suxamethonium). The advisability of allowing upper respiratory tract interventions by inexperienced personnel in patients under deep halothane anaesthesia without a secure and protected airway may be questioned. Concern has been expressed recently about a report that oxygen desaturation, airway obstruction, hypoventilation, laryngeal spasm, hypotension, bradycardia and other complications occurred when investigators used a similar anaesthetic technique [19, 20], although they elected not to use neuromuscular block for the last part of the procedure. We have described a graduated training programme designed to avoid the possibility of inadequate ventilation of the lungs and cardiovascular depression. The process of fibreoptic intubation is subdivided into the elements of endoscopy and intubation. Initially, fibreoptic endoscopy is practised on intubated, ventilated patients under light anaesthesia with stable cardiovascular and respiratory physiology. Trainees are required to demonstrate considerable expertise in endoscopy before embarking on fibrescope-guided intubations of apnoeic patients.

One of the main problems of traditional fibreoptic training is that only one person can visualize the procedure at any moment. Hence, trainees cannot follow the instructor's demonstrations and the instructor cannot respond effectively to the trainee's difficulties. Verbal guidance in this context is poorly targeted and imprecise. As a result, novices learn largely by a process of trial and error and can easily become discouraged because of lack of progress. Figure 3 shows the progress record of a typical member of the traditional group. During the first stage of motor learning, the verbal-cognitive stage [21], the main concern is to understand what needs to be done and to decide which is the best way to do it. Early endoscopies are protracted and there are several "failures" as he experiments with different

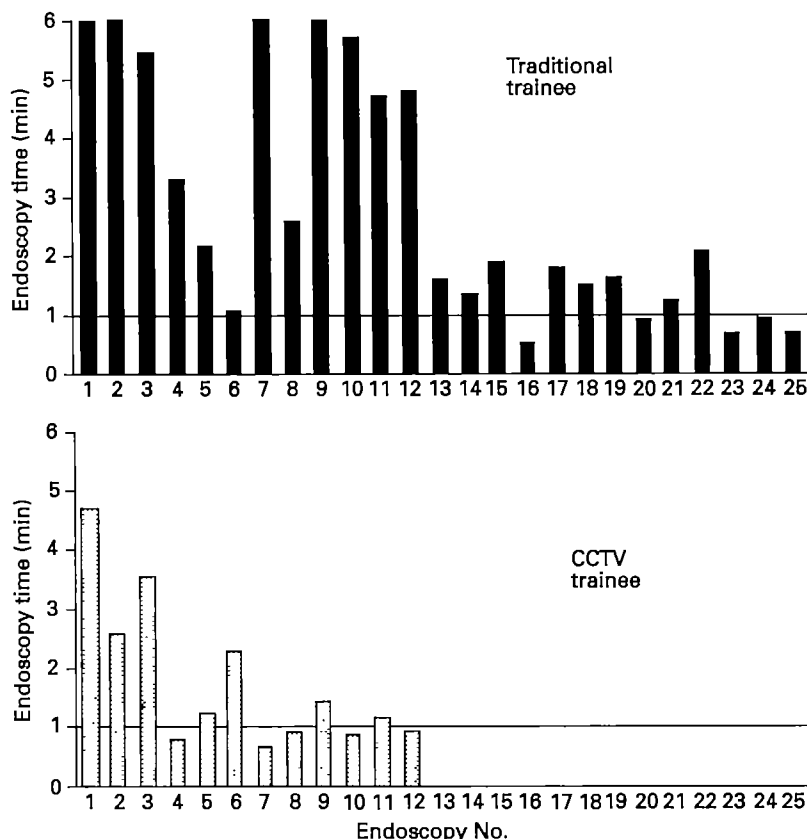


FIG. 3. Progress records of typical traditional group trainee and typical CCTV group trainee during stage 3 (endoscopies).

strategies. After 10 to 12 endoscopies, this particular trainee has solved many of the cognitive problems and appears to have mastered a satisfactory technique. As the trainee passes through the second stage of learning, the motor stage, better movement patterns are developed to produce the desired actions. Advancement is now more rapid as efficiency and consistency improve.

The CCTV system, used in the way we have described, appears to double the rate of acquisition of fiberoptic intubation skills. It does this by enabling the instructor to make a more positive and direct teaching contribution; in particular, it facilitates demonstrations and feedback [22]. Video demonstrations transmit practical skill information far more easily than verbal descriptions. Extrinsic feedback is information provided (fed back) by the instructor to the trainee, in response to practice activities. It enables errors to be detected accurately and corrected quickly and difficulties to be discussed and resolved. Trainees in the video group usually pass through a very short verbal-cognitive stage. Typically, they grasp the essential rules and strategies during the initial session conducted on the airway model and, with this background, soon begin to organize competent movement patterns. Figure 3 also shows the progress record of a typical member of the video group. By the time trainees start endoscopy practice with patients, they are already well into the process of consolidating motor skills. Few protracted endoscopies are observed and the rehearsal of faulty and ineffectual strategies is kept to a minimum. As proficiency improves, however, it is important that

the instructor does not overdo feedback guidance, as it is known to possess dependence-inducing properties [10]. The aim should be to allow trainees to develop their own error detection capabilities and intrinsic feedback, so that they can perform independently. Thus extrinsic feedback should be faded and then stopped with advancing skill. In this regard, it might be advantageous for trainees to change to traditional endoscopy practice earlier than in the design of this study, for example, as soon as they are consistently completing endoscopies in less than 2 min.

The third stage of motor learning is the autonomous stage, which is characterized by refined, automatic movements that demand little attention. This stage develops slowly and requires a great deal of practice. Perhaps several hundred endoscopies/intubations under the most favourable conditions are necessary before automatic action patterns emerge. At the end of this clinical investigation, the trainees had certainly not achieved this level of expertise. However, they had reached a safe, intermediate level of ability. They are now in a good position to improve and extend their skills by using the fiberoptic laryngoscope as an alternative to the Macintosh laryngoscope in suitable routine patients, provided they continue to receive appropriate supervision and audit review.

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RESPIRATORY SINUS ARRHYTHMIA: AN INDEX OF LIGHT ANAESTHESIA

C. J. D. POMFRETT, J. R. BARRIE AND T. E. J. HEALY

SUMMARY

This study was designed to test if changes in the degree of respiratory sinus arrhythmia (RSA) can be used as an index of light anaesthesia. An on-line, real-time data logging system was used to record simultaneously the EEG and ECG waveforms from 10 patients undergoing routine surgery using i.v. propofol (Diprivan) anaesthesia. The degree of RSA was determined in real-time. The median frequency of the EEG was derived off-line and correlated with the degree of RSA. Time series analysis was performed on the derived indices off-line. Significant changes in the degree of RSA occurred in response to changes in propofol infusion in all patients; these changes corresponded also to changes in the median frequency of the EEG. Heart rate and ventilatory frequency are measured routinely during surgery and it is suggested that on-line monitoring of RSA, derived from these standard signals, provides a more convenient and objective index of lightening anaesthesia than either EEG analysis or classical estimates of anaesthetic depth based on arterial pressure or heart rate. (Br. J. Anaesth. 1993; 71: 212–217)

KEY WORDS

Anaesthesia: depth. Heart: heart rate variability, electrocardiography.

Established, subjective assessments of anaesthetic depth such as the pupillary light reflex, lachrymation, sweating and movement are suppressed by modern neuromuscular blocking drugs and opioid analgesics. Attempts have been made, therefore, to develop an objective, reliable index of anaesthetic depth in order to ensure that inadvertent awareness does not occur during surgery as a result of inadequate anaesthesia [1–4]. Several putative indices have been proposed, including derived indices of the EEG, auditory evoked potentials (AEP), the frontalis electromyogram (FEMG), and lower oesophageal contractility (LOC). Each method relies on opening a window on activity in the brain by measuring the effect of anaesthetic agents on different sensory or motor pathways. However, none of these methods has achieved wide acceptance by anaesthetists: for example, EEG equipment is complex, costly and may be subject to artefact from

common procedures such as diathermy, and EMG signals are suppressed by large doses of neuromuscular blocking agents.

Other aspects of central nervous activity have not been studied extensively during anaesthesia. Respiratory sinus arrhythmia (RSA), a non-pathological variation in heart rate with breathing, has been used as a method of screening diabetic patients exhibiting autonomic neuropathy [5]. The degree of RSA reflects the parasympathetic tone present as inhibition of the heart by the vagus, and is mediated by activity in the brainstem. One off-line study during isoflurane anaesthesia has suggested that on-line monitoring of RSA may provide an index of anaesthetic level [6]. We have demonstrated already that changes in the degree of RSA during recovery from isoflurane anaesthesia reflect awakening in all paralysed patients undergoing mechanical ventilation of the lungs [7]. Spectral analysis of heart rate variability during propofol induction has demonstrated that RSA diminishes during anaesthesia [8]. Measurements of heart rate variability are particularly suited to routine use in the operating theatre, as minimal additional instrumentation is required, in contrast with techniques based around the auditory evoked potential or EEG recording.

Although it has been demonstrated that patients can be awakened ethically immediately after induction of anaesthesia, but before surgery commences [9], it is clearly not feasible to rouse patients deliberately during the course of potentially painful surgical procedures in order to test the effectiveness of putative warnings of light anaesthesia. However, it is possible to record accurately the times of events known to reduce anaesthetic depth, such as incision or accidental removal of an anaesthetic agent, at the same time as recording established indices of anaesthetic depth. The objective of this study was to test if RSA could give a warning of impending light anaesthesia during propofol anaesthesia.

PATIENTS AND METHODS

We examined 10 female ASA I patients undergoing elective gynaecological surgery under general anaesthesia (mean age 34.4 yr, range 30–45 yr; mean

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weight 65.1 kg, range 42–91 kg). Each patient gave informed, written consent to the study, which was approved by the local Ethics Committee.

The equipment used for this study has been described briefly elsewhere [10]. Ventilatory flow was recorded using a Magtrak turbine (Ferraris Medical Ltd). Electrocardiogram (ECG) and ventilatory flow data were collected using a Digitimer Neurolog system, 12-bit digitized at 1-KHz sampling frequency (Cambridge Electronic Design 1401), and analysed on-line, in real-time by a 32-bit reduced instruction set microcomputer (Acorn Archimedes) using multitasking software developed by us for this study. Data acquisition was uninterrupted by analysis or display of results. Patients were requested to breathe into a face mask attached to the ventilatory flow meter for 5 min before anaesthesia while their ECG was monitored. This allowed baseline RSA to be recorded.

Patients were premedicated with temazepam 20 mg 90 min before operation. Infusion rates of propofol and notes made by the anaesthetist were logged into an event-marking database, so that precise event timing could be examined off-line. The anaesthetic technique was similar to that described elsewhere [11]. Anaesthesia was induced with propofol (2.5 mg kg^{-1} bolus) and maintained with 66% nitrous oxide in oxygen, fentanyl $3 \text{ } \mu\text{g kg}^{-1}$ and a continuous infusion of propofol ($10 \text{ mg kg}^{-1} \text{ h}^{-1}$ for the first 10 min then $8 \text{ mg kg}^{-1} \text{ h}^{-1}$ for 10 min and $6 \text{ mg kg}^{-1} \text{ h}^{-1}$ for subsequent anaesthesia). The doses of propofol and fentanyl were increased when light anaesthesia was detected by the anaesthetist during surgery. Vecuronium 0.1 mg kg^{-1} was used to provide neuromuscular block in all patients during controlled ventilation. Measurements were collected continuously with brief interruptions at tracheal intubation and transfer of the patient from the anaesthetic room to the operating theatre, and occasional archiving of data from fixed disc to tapestreamer. Data were collected until patients opened their eyes or moved in response to spoken commands. The trachea was then extubated. Residual neuromuscular block was antagonized with neostigmine and glycopyrronium.

EEG recording was used to provide an objective estimate of anaesthetic level. Silver–silver chloride cup electrodes were attached to skin treated with a preparatory paste (Omni-Prep, Weaver & Co.) using electrode adhesive (Ten 20, Weaver & Co.). Frontal (F_z) and mastoid locations were chosen, with a shoulder ground, as these positions resulted in minimal delay to the normal function of the anaesthetic room. Electrode impedance was maintained at less than $5 \text{ K}\Omega$ throughout the procedure. The electrodes were attached to isolated preamplifiers (Digitimer Neurolog) and subject to analog bandpass filtering (0.1–50 Hz). The EEG was sampled with 1-KHz and 12-bit digitization. Software developed for the study performed a fast Fourier transform on 6-s epochs of the sampled EEG (2048 points) and derived a spectral array for each epoch. Compressed spectral arrays, median and edge frequencies could be derived from these data off-line.

An anaesthetic gas analyser (Datex, Capnomac Ultima-1) was used to allow standardized end-tidal concentrations of nitrous oxide and carbon dioxide to be maintained. Paper traces of trended systolic pressure and arterial oxygen saturation were obtained from theatre monitors. No computerized arterial pressure or oxygen saturation records were collected and so no statistical analysis of these indices was possible.

Statistical analysis

Circular statistical analysis of the ECG and ventilation was performed in order to derive the degree of RSA [12]. R-waves were extracted from the ECG using amplitude discrimination and plotted relative to their time on a normalized unit ventilatory waveform on-line and in real-time. The degree of clustering about this unit waveform was assessed using circular statistics whereby each ECG R-wave was resolved as a vector with an amplitude of 1 and an angle representing the position of the ECG R-wave within the ventilatory cycle. The mean vector length (R) was calculated at 1-min intervals, and presented as a number ranging from 0 to 1, depending on the degree of RSA. R for each sample was presented as a graph against time on-line to anaesthetic staff in the operating theatre, and all raw data were archived. Changes in RSA were indicated as changes in the value of R . Significant differences from previous techniques used to measure RSA based on circular statistics [13] were that the unit ventilatory waveform was normalized on-line from breath to breath with R , and ventilatory events determined in real-time over consecutive minutes under the control of the anaesthetist. Measurements could then be made on subjects breathing spontaneously or undergoing controlled ventilation, without the necessity for a standard breathing frequency or heart rate.

Rayleigh's test for randomness was applied to data on-line. Increases in R to greater than the test statistic for Rayleigh's test ($P = 0.05$) showed that the change in the degree of RSA was significant. The test statistic was calculated from the number of ECG R-waves in a sampling epoch, and was presented on-line on a graph as a red line, with R shown as a green line on the same axes. It was, therefore, easy for the anaesthetic staff to see if a significant change in R had occurred during the most recent epochs of data collection.

In order to compare EEG and RSA data, smoothing was performed on each time series using appropriate, non-seasonal linear or exponential models. Autocorrelation was performed on residuals from the fitted data in order to ensure that appropriate models had been applied. Autoregressive Integrated Moving Average (ARIMA) analysis was performed off-line on time series R data before and after interventions in the time series caused by changes in propofol infusion rate or other events noted by the anaesthetist. Control charts were prepared and significant variations in the degree of RSA from predicted ARIMA models were noted ($P < 0.01$). Analysis was performed using a standard

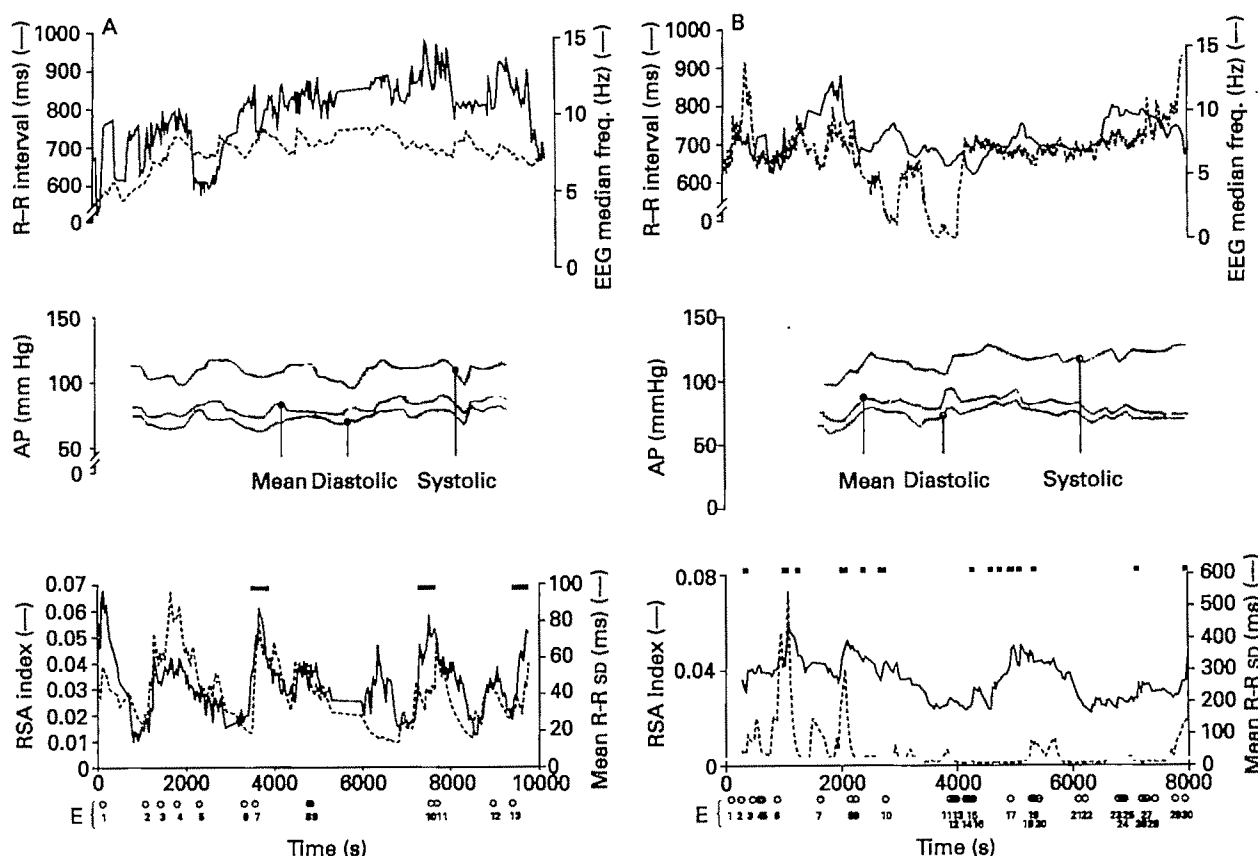


FIG. 1. Representative time courses of mean ECG R-R interval, median frequency of the EEG, arterial pressure, (AP) respiratory sinus arrhythmia (RSA) index and SD from the mean ECG R-R interval during anaesthesia. Symbol $\odot$ denotes events. A Events: 1 = propofol induction; 2 = vecuronium; 3 = incision; 4 = propofol $8 \text{ mg kg}^{-1} \text{ h}^{-1}$; 5 = fentanyl; 6 = propofol off; 7 = propofol $6 \text{ mg kg}^{-1} \text{ h}^{-1}$; 8 = propofol off; 9 = propofol $6 \text{ mg kg}^{-1} \text{ h}^{-1}$; 10 = increase in heart rate noted by anaesthetist; 11 = 40-mg bolus of propofol; 12 = closing stitches; 13 = propofol off. B Events: 1 = start record; 2 = eyes closed; 3 = start propofol induction; 4 = 50-mg propofol bolus; 5 = intubation; 6 = ventilator on; 7 = complete transfer to operating theatre; 8 = propofol $10 \text{ mg kg}^{-1} \text{ h}^{-1}$; 9 = incision; 10 = observation from surgeon: "muscle tone"; 11 = tracheal tube cleared; 12 = propofol off; 13 = propofol on; 14 = suction to clear airway; 15 = reconnect tracheal tube; 16 = 20-mg propofol bolus; 17 = propofol $8 \text{ mg kg}^{-1} \text{ h}^{-1}$; 18 = disconnection of airway; 19 = reconnect airway; 20 = aminophylline given in response to bronchospasm; 21 = propofol cannula cleared; 22 = propofol $6 \text{ mg kg}^{-1} \text{ h}^{-1}$; 23 = propofol off; 24 = propofol on; 25 = propofol $6 \text{ mg kg}^{-1} \text{ h}^{-1}$; 26 = surgeon stitching; 27 = propofol off; 28 = stitching completed; 29 = clearing airway; 30 = eyes opened.

statistical package [14] running on an AT-compatible personal computer.

RESULTS

No patient had explicit recall of intraoperative events. Representative time series of R and the median EEG frequency are shown in figure 1. We observed increases in the degree of RSA recorded on-line when anaesthesia was discontinued for more than 1 min in all 10 patients. This increase in RSA was accompanied by a corresponding increase in median EEG frequency whenever the median frequency was originally less than 10 Hz. We suggest, therefore, that increases in the degree of RSA accompany lightening of propofol anaesthesia. In addition, a slower reduction in the degree of RSA was observed in all patients on deepening anaesthesia, which was accompanied also by a corresponding reduction in the median frequency of the EEG. We observed that the changes in the degree of RSA always preceded changes in the EEG.

Off-line ARIMA modelling of R showed it to increase significantly from predicted values in all

patients when anaesthesia lightened after the propofol infusion was discontinued ($P < 0.01$). An example of this increase in R seen during an inadvertent interruption of the propofol infusion is shown in figure 2 (data of figure 2 were extracted from the same record as figure 1A). The anaesthetist recharged the propofol syringe, but did not switch the infusion pump back on until 4 min 11 s had elapsed, when it was realized that the pump was off. R increased rapidly and was detected on-line. There was also an increase in heart rate (from 73 to 83 beat min^{-1}), but no other clinical signs of light anaesthesia were noted before i.v. anaesthesia was recommenced (fig. 1A, events 6–7). Off-line ARIMA analysis showed that R exceeded the 99% confidence limits for the predicted behaviour of R based on its behaviour over the previous 15 min and this was, therefore, a very significant increase in the degree of RSA (fig. 2A). The median frequency of the EEG was variable but increased from around 7 Hz to 9 Hz over the same time course. The propofol syringe was recharged again 25 min later, but this time the infusion pump was switched back on only 31 s after it was switched off. R did not increase significantly in

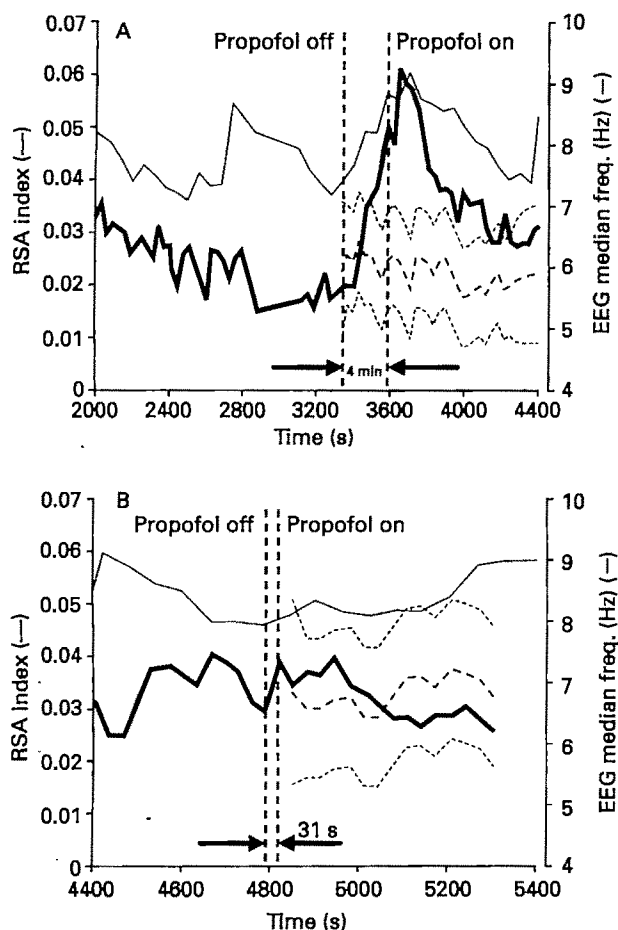


FIG. 2. Time course of changing respiratory sinus arrhythmia (RSA index) (—) and median EEG frequency (---) during transient interruption of anaesthetic infusion: detailed extracts from figure 1A, with additional ARIMA control data. A: Effect of a 4-min interruption in propofol infusion because of inadvertent failure to switch the propofol syringe pump on for 4 min after recharging the propofol supply. Note the large increase in RSA index (R) which clearly exceeds the 99% confidence limits (---) predicted for the behaviour of R , based on the previous 15 min of data. B: More normal, non-significant change in R associated with a 31-s interruption in propofol infusion. --- = predicted R (ARIMA analysis).

response to such a short interruption in the propofol infusion (fig. 2B). The median frequency of the EEG similarly did not increase in response to this brief cessation of anaesthesia.

In addition, R increased significantly in two patients on incision. These patients were subsequently shown to have high median EEG frequencies in response to incision (> 8 Hz), which suggests that they were lightly anaesthetized.

Figure 3 shows the results from analysis of grouped data from all 10 patients. Two 1-min samples (four 30-s data points) were taken from the time series data, with a 1-min delay (two 30-s data points) after a change in anaesthetic level, to allow the anaesthetic to take effect. There was clearly a trend in the mean R values whereby increasing levels of anaesthesia resulted in smaller values of R . No such trend was visible in the grouped median EEG frequency data. Neither the median frequency EEG nor the R data exhibited a normal distribution. Non-parametric statistics (Wilcoxon rank sum) were used, therefore, to determine if there was a significant

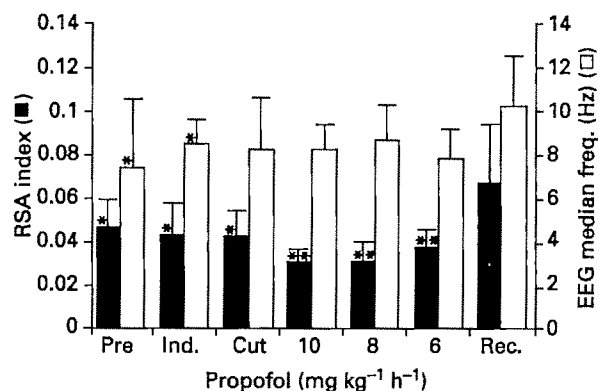


FIG. 3. Bar graph showing group data analysis from 10 patients in this study. Each bar represents the mean (SEM) for 2-min samples of RSA index and median frequency of the EEG, taken 1 min after each event. The 1-min delay before sampling was chosen to allow time for the change in drug concentration to take effect. Pre = Before induction; Cut = incision; Rec = recovery. Significant differences compared with recovery: * $P < 0.05$; ** $P < 0.01$ (non-parametric tests).

difference between the distribution of the samples from the different levels of anaesthesia. These statistics were used after reference to an earlier study to test the effectiveness of measuring anaesthetic level using lower oesophageal contractility [9]. In this previous study, the investigators had awakened patients after induction, and they compared lower oesophageal contractility before and after recovery. We also, therefore, compared values of R and the median EEG frequency both before and after recovery, and additionally compared different levels of anaesthesia. We observed significant differences between R at all levels of anaesthesia compared with that after anaesthesia was discontinued ($P < 0.01$ for propofol 10 mg kg⁻¹ h⁻¹, 8 mg kg⁻¹ h⁻¹ and 6 mg kg⁻¹ h⁻¹; $P < 0.05$ after induction and incision). We observed a significant difference between the median frequency of the EEG only at recovery and induction ($P = 0.023$), with no significant differences between other levels of anaesthesia and the recovery value. We also observed significant differences between both R ($P = 0.032$) and median EEG frequency ($P = 0.021$) on comparing samples taken before induction and after recovery from anaesthesia.

DISCUSSION

We suggest that on-line monitoring of RSA using the derived index, R , is a convenient new tool for the detection of light anaesthesia. We further suggest that detailed time series analysis of RSA on-line, which we are developing as a result of our off-line analysis presented here, provides an indicator of depth of anaesthesia. It has been suggested that changes in heart rate variability correlate with changes in the EEG during anaesthesia [15], one of the conclusions of that study being that cortical electrical activity and heart rate variability are probably controlled by the same, common sub-cortical influence of anaesthesia. RSA monitoring possesses several important advantages over EEG monitoring: RSA indices are derived from conventional, easily recorded, ECG and ventilatory

signals, whereas the EEG requires at least two scalp electrodes; EEG amplifiers are costly and EEG signals are of a low amplitude, making them susceptible to contamination by electrical artefacts introduced by other electrical apparatus including diathermy, whereas ECG signals are relatively large and easily screened from these artefacts; the latency of change in RSA on discontinuing anaesthesia appears consistently shorter than that seen in the EEG on discontinuing anaesthesia, resulting in a more rapid indication of potentially dangerous light anaesthesia.

We noted that the median EEG frequency decreased to less than 5 Hz only occasionally during higher levels of propofol anaesthesia (e.g. figure 1A induction and figure 1B $10 \text{ mg kg}^{-1} \text{ h}^{-1}$). These values are greater but, after correction for the mean weight-related dosage given in our study, are still generally within 1 SD of the average median EEG frequency given in a prior study of propofol anaesthesia in seven volunteers reported by Schwilden [16]. From values quoted in the earlier study, we would have expected our EEG median frequencies to be: $5 \text{ Hz} \pm 2 \text{ Hz}$ at 6.51 mg min^{-1} ; $3 \text{ Hz} \pm 2 \text{ Hz}$ at 8.15 mg min^{-1} ; and $2.5 \text{ Hz} \pm 0.5 \text{ Hz}$ at 10.9 mg min^{-1} . It is possible that the greater values of EEG median frequency in our study can be attributed to the presence of strong surgical stimulation. Our grouped EEG data frequencies (fig. 3) were considerably greater than the frequencies reported by Schwilden, with the only significant differences between dosages based on the median frequency of the EEG being those between recovery and induction and pre-induction values. The reason for this is the long latency, of about 3 min, between changes in anaesthetic level causing a change in the median EEG frequency. For the grouped analysis, we extracted samples from the median EEG and the *R* records with epochs of 2 min, beginning 1 min after the change in depth of anaesthesia. With these epochs, the level of *R* clearly changed significantly, but the median frequency of the EEG did not change. This result suggests that circular statistical determination of respiratory sinus arrhythmia responds to changes in the depth of propofol anaesthesia faster than the median frequency of the EEG.

Any study using the median frequency of the EEG during anaesthesia is limited by delays in the effect of anaesthetic agents, either on the cerebral cortex, or on ascending pathways mediating activation of the thousands of pyramidal neurones, the combined activity of which is recorded at the scalp as the EEG. Any fast-Fourier transform-based acquisition system requires a reasonable time in which to process the raw EEG data, and this imposes a further delay on the temporal resolution of the system. In contrast, circular statistical analysis of RSA, as performed in this study, requires only a 30-s epoch to give immediate indications of activity from vagal output of the nucleus solitarius of the brainstem. Studies on the time-course of propofol uptake in the brainstem in contrast to uptake in the higher cerebral centres have yet to be performed, but it seems reasonable to propose that a fundamental, brainstem-mediated

ventilatory reflex (respiratory sinus arrhythmia) is affected by anaesthetic agents more rapidly than the cortical EEG.

RSA has been distinguished clearly from lower frequency baroreceptor reflexes by the use of spectral analysis of heart rate variability [8]. We were unable to compare the effect of gross changes in arterial pressure statistically against RSA, but we suggest that these effects would be most pronounced in the medium or low frequency components of heart rate variability, which are not detected using circular statistical analysis, as this specifically identifies the RSA component of heart rate variability. This suggestion is supported by visual inspection of our arterial pressure records in comparison with RSA (fig. 1), in which there is clearly no clear correlation between RSA and arterial pressure, although it is expected that an increase in pressure associated with light anaesthesia may be accompanied by an increase in RSA.

In order to develop a reliable indication of depth of anaesthesia, any putative technique has to meet several criteria [17]: the technique should indicate the stage during light anaesthesia that precedes conscious awareness and, preferably, overt clinical responses; the output should reflect increasing and decreasing concentrations of anaesthetic and be sensitive to stimuli presented to the patient, of which surgical stimulus is the most important; the method should have a high temporal resolution; it should be capable of staging the depth of anaesthesia. Our on-line monitor of RSA currently meets three of these four criteria: it is capable of reliably detecting the transition to and from light anaesthesia; it detects the arousal present in lightly anaesthetized patients as a result of surgical stimulation; it has a temporal resolution of 30 s which, with appropriate windowing techniques, has been reduced off-line to 6 s. Off-line ARIMA analysis has allowed us to stage the depth of anaesthesia and hence meet the fourth criterion, which we are presently implementing in an on-line monitoring system. One further advantage of the present system is that the simple, two-line display is easily understood by theatre staff and needs no calibration between patients.

It is necessary to examine changes in many hundreds of patients under different anaesthetic regimes in order to validate RSA as a method for warning of light anaesthesia and, ultimately, as a method of determining the depth of anaesthesia. In addition to propofol, we have now studied the effects of three other i.v. anaesthetics (etomidate, thiopentone and methohexitone) and the volatile agents isoflurane, halothane and enflurane, and will report the characteristics of the changes in RSA in other communications [7 and in preparation]. We are also investigating the effects of drugs such as atropine on RSA, which were not used in this study. Other research suggests that pharmacological doses of atropine, as used routinely at reversal of paralysis, do not abolish RSA [18].

The implications of on-line RSA monitoring for automated drug delivery have already been discussed with reference to agents acting on the parasympathetic nervous system [19]. The fast response

time of changes in RSA on changing concentrations of i.v. propofol shown in the present study suggest that it would be suitable as a technique for the automated control of i.v. anaesthetic agents, as these drugs have a rapid effect which needs fast, responsive and accurate physiological feedback in order to control an integrated delivery system, whereas volatile anaesthetic agents have a slower recovery time.

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NATURAL KILLER CELL ACTIVITY AND LYMPHOCYTE SUBPOPULATIONS DURING DOBUTAMINE INFUSION IN MAN

Y. NOMOTO, H. JHONOKOSI AND S. KARASAWA

SUMMARY

We have studied the effects of dobutamine on natural killer (NK) cell activity and the distribution of lymphocyte subpopulations in peripheral blood in 16 patients under general anaesthesia. Patients were allocated to receive either dobutamine ($n = 9$) or isotonic saline ($n = 7$) for 30 min. There was a significant decrease in the ratio of T-helper/inducer cells (CD4) to T-suppressor/cytotoxic cells (CD8) in all patients after induction of anaesthesia. Dobutamine infusion induced an increase in NK cell activity, accompanied by a selective increase in circulating NK cells. However, compared with preinfusion values, CD4:CD8 cell ratio did not change during and after dobutamine infusion. In the isotonic saline group, NK cell activity and CD4:CD8 cell ratio remained unchanged. These results suggest that dobutamine induced an increase in NK cell activity and alterations in lymphocyte subpopulations. (Br. J. Anaesth. 1993; 71: 218-221)

KEY WORDS

Immune response: natural killer cell activity. Pharmacology: dobutamine.

NK lymphocytes are a subset of lymphocytes exerting spontaneous cytotoxicity against a variety of tumour cells. NK cells have been shown in mice to play a role in primary immune defence against tumour formation and defence against metastatic spread of tumours. Human NK lymphocytes have been identified and shown to be cytotoxic to human tumour target cells *in vitro* [1].

NK cell activity is enhanced after premedication and during anaesthesia and surgery [2], followed by a reduction for 3-6 days after major surgery [3, 4].

Several studies have shown that administration of adrenaline to human subjects produces an increase in NK cell activity [5]. This change is prevented by simultaneous incubation with the beta-adrenoceptor antagonist, propranolol [6]. It has been suggested that adrenaline pretreatment enhances NK cell activity via lymphocyte beta-adrenoceptors. There have been no reports to date on NK cell activity after administration of dobutamine to human subjects.

PATIENTS AND METHODS

With local Ethics Committee approval and informed patient consent, we studied 16 adult patients with

lumbar disc herniation undergoing elective partial laminectomy. The patients were otherwise healthy with normal cardiac, renal and hepatic functions; none had endocrine disorders and none was receiving any medications. The patients were allocated randomly to receive either dobutamine ($n = 9$) or isotonic saline ($n = 7$) (table I).

Premedication and general anaesthesia were identical for all patients: premedication comprised atropine 0.5 mg and hydroxyzine 50 mg i.m. 1 h before operation; anaesthesia was induced with thiopentone 5 mg kg⁻¹ and intubation was performed after administration of vecuronium 0.16 mg kg⁻¹. Anaesthesia was maintained by artificial ventilation with enflurane and 66 % nitrous oxide in oxygen. In all patients, i.v. infusions during surgery were restricted to Ringer lactate solution at a rate of 10 ml kg⁻¹ during the first 1 h and thereafter at 2-4 ml kg⁻¹ h⁻¹.

After steady-state conditions were established, the patients were allocated randomly to two groups to receive a continuous i.v. infusion of either dobutamine 5 µg kg⁻¹ min⁻¹ or isotonic saline, for 30 min.

TABLE I. Patient data (mean (range or SD))

	Group 1 (dobutamine)	Group 2 (isotonic saline)
No. patients	9	7
Sex (M/F)	7/2	6/1
Age (yr)	25 (16-45)	29 (20-43)
Weight (kg)	66 (9)	63 (10)
Duration of op. (min)	146 (48)	163 (55)

TABLE II. Schedule of blood sampling

Sample No.	Sampling time
1	Before induction of anaesthesia
2	Before infusion of dobutamine or isotonic saline
3	15 min after beginning of infusion
4	30 min after beginning of infusion
5	30 min after stopping infusion
6	90 min after stopping infusion

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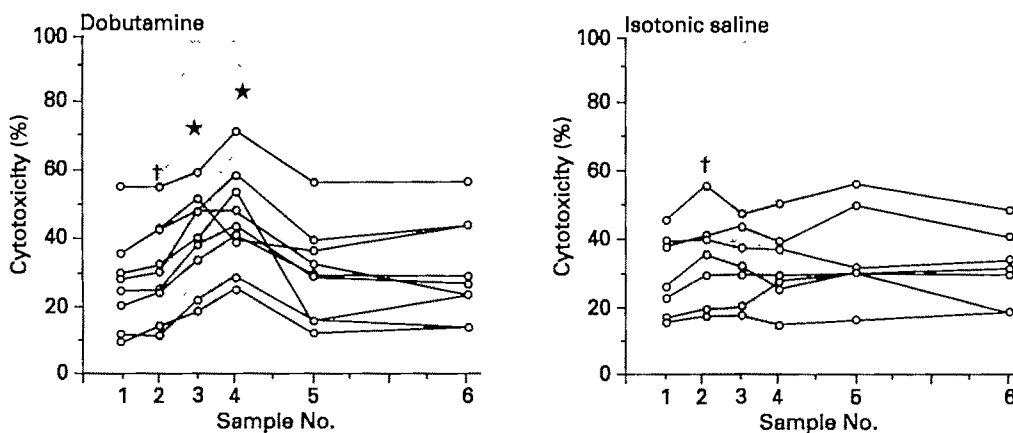


FIG. 1. Individual changes in NK cell activity expressed as % cytotoxicity before, during and after infusion of dobutamine or isotonic saline. Shaded area represents period of infusion. Sample Nos: 1 = Before induction; 2 = before infusion; 3, 4 = 15, 30 min after beginning of infusion; 5, 6 = 30, 90 min after stopping infusion. $P < 0.05$: †compared with sample 1; *compared with sample 2.

Haemorrhage did not exceed 200 ml and blood substitutes or sympathomimetic agents were not required.

Arterial blood samples were obtained as shown in table II, for measurement of NK cell activity, distribution of lymphocyte subpopulations, blood leucocyte counts and measurement of serum cortisol, plasma adrenaline and noradrenaline concentrations. Plasma adrenaline and noradrenaline concentrations were measured by high pressure liquid chromatography, serum cortisol by radioimmunoassay.

The pan T-cell population (CD3) was measured with Leu4 antibody, the T-helper/inducer cells (CD4) with Leu3a antibody, the T-suppressor/cytotoxic cells (CD8) with Leu2a antibody and NK cells (CD16) with Leu11 antibody (Becton Dickinson Monoclonal Center, Mountain View, CA, U.S.A.) using flow cytometry (Ortho Spectrum III, Ortho Diagnostic Systems Inc.).

NK cell activity was measured against K-562 target cells in a chromium-51 release assay. Five $\times 10^5$ ^{51}Cr -labelled target cells of the human erythroleukaemic cell line K562 were mixed with different concentrations of mononuclear cells from patients, for use as effector cells, to obtain effector to target cell ratios 100:1, 50:1, 25:1 and 12:1. Cell suspensions were incubated at 37 °C in humidified air and 5% carbon dioxide for 4 h. After incubation, the radioactivity of 100 μl of the cell-free supernatant was counted with an automatic well-type gamma counter. Percentage cytotoxicity was computed from the formula:

% cytotoxicity =

$$\frac{(\text{experimental release} - \text{spontaneous release})}{(\text{maximum release} - \text{spontaneous release})} \times 100$$

Spontaneous chromium-51 release was measured by incubating target cells with assay medium, and maximum release was measured by incubating target cells in water containing 5% sodium sulphate.

Data are expressed as mean (SD). Statistical analyses were performed using the Wilcoxon test. The level of significance was defined as $P < 0.05$.

RESULTS

There was no difference in age, weight or duration of surgery between the two groups (table I).

Individual changes in NK cell activity before, during and after infusion of dobutamine or isotonic saline are shown in figure 1.

A significant increase in NK cell cytotoxicity occurred throughout the infusion of dobutamine, and this returned to pre-infusion values 30 min after completing infusion. In the control group, NK cell cytotoxicity did not change significantly during and after the infusion. After induction of anaesthesia, a small but significant increase in NK cell cytotoxicity occurred in both groups.

The numbers of circulating leucocytes, lymphocytes and the pan T-cells (CD3) and NK cells (CD16) are shown in figure 2. In the dobutamine group, absolute leucocytosis and lymphocytosis occurred. The pan T-cells showed no significant change, but the number of NK cells increased during and after the infusion of dobutamine. In the control groups, the number of NK cells did not change significantly.

The ratio of CD4 and CD8 cells was significantly reduced after induction of anaesthesia in all patients (fig. 3). However, the ratio did not change during and after infusion of either dobutamine or saline.

In both groups, serum cortisol, plasma adrenaline and noradrenaline concentrations did not change significantly throughout the study.

In the dobutamine group, mean systolic arterial pressure increased from 110 mm Hg (range 85–145 mm Hg) to a peak value of 130 mm Hg (range 115–155 mm Hg) ($P < 0.05$), but there were no significant changes in diastolic arterial pressure. Heart rate also increased, from 73 to 85 beat min^{-1} , in the dobutamine group. In the isotonic saline group, neither arterial pressure nor heart rate changed significantly during the study.

DISCUSSION

We have found that dobutamine selectively increased NK cell activity and increased the absolute number of circulating NK cells *in vivo*.

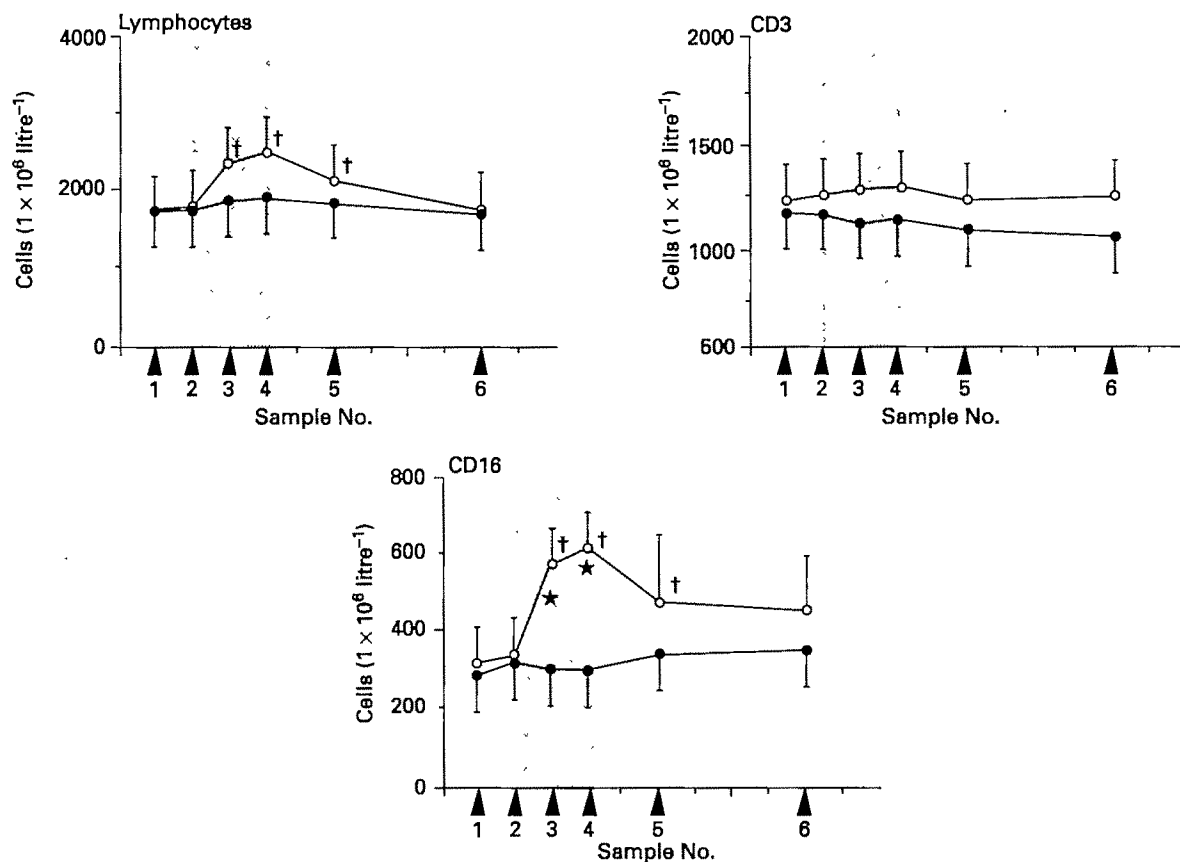


FIG. 2. Number of circulating lymphocytes: pan T cells (CD3) and NK cells (CD16) before, during and after infusion of dobutamine (O), or isotonic saline (●). Shaded area represents period of infusion. Sample Nos as in figure 1. $P < 0.05$: †compared with sample 2; *compared with isotonic saline.

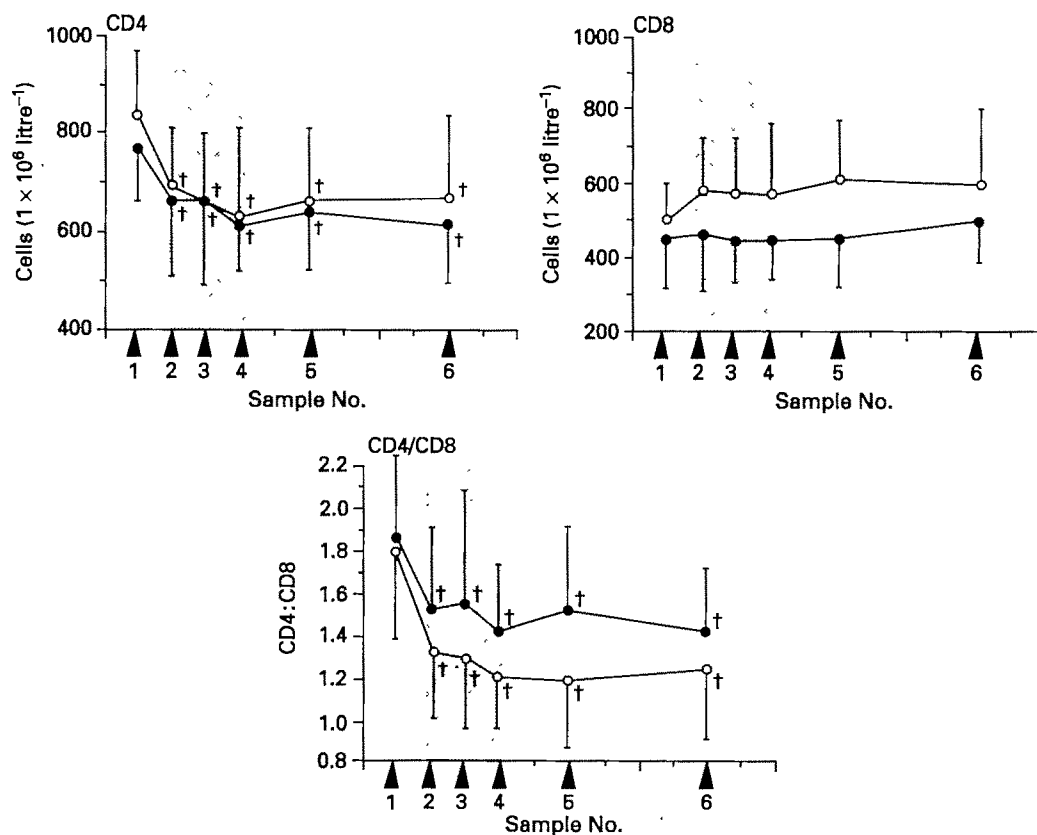


FIG. 3. Number of T-helper/inducer cells (CD4), T-suppressor/cytotoxic cells (CD8) and CD4:CD8 ratio in peripheral blood (mean, sd) before, during and after infusion of dobutamine (O) or isotonic saline (●). Shaded area represents period of infusion. Sample Nos as in figure 1. † $P < 0.05$ compared with sample 1.

This increase in NK cell activity was consistent with changes observed in other studies, after administration of adrenaline [5, 6]. Tønnesen, Mickley and Grunnet observed that, after administration of adrenaline s.c. to healthy individuals, NK cell activity increased significantly within 15–30 min [2]. It has been shown also that the percentage of NK cells in peripheral blood increased after administration of adrenaline [5]. This enhancement of NK cell activity correlated with an increased number of circulating NK cells during operation.

Dobutamine is a potent adrenergic agonist. There have been no reports describing NK cell activity after administration of dobutamine to humans. Circulating human lymphocytes possess a large number of beta-adrenoceptors [6], but the biological significance of this is unknown.

Hellstrand demonstrated that adrenaline enhanced NK cell activity *in vivo*. The increased activity was prevented consistently by simultaneous incubation with the non-selective beta-adrenoceptor antagonist, propranolol [6]. Thus it may be assumed that adrenaline pretreatment enhanced NK cell activity via lymphocyte beta-adrenoceptors. Adrenaline-induced beta-adrenoceptor activation is believed to increase intracellular cAMP via the adenylate cyclase complex. Similar results have been obtained with prostaglandin E₂, a potent inducer of intracellular cAMP [7], which was shown to enhance NK cell activity in low, physiological concentrations.

Although the mechanism of lymphocyte release is not known, our data clearly demonstrate that dobutamine altered the distribution of circulating subsets. The rapid increase in NK cells may be explained by mobilization of mature NK cells from the extravascular space into the circulation or by differentiation of pre-NK cells to CD16 mature cells [2]. Adrenaline may cause this "mobilization" of lymphocytes directly, by activation of lymphoid adrenergic receptors, or indirectly, by activation of adrenergic receptors in other tissues.

The human immune system is regulated, in part,

by interacting networks of helper/inducer (CD4) and suppressor/cytotoxic (CD8) T cells. The ratio of CD4 cells to CD8 cells, a commonly used indicator of immunoregulatory cell imbalance, was altered after induction of anaesthesia in our study. Although the absolute number of CD8 cells remained unchanged after induction of anaesthesia, the CD4:CD8 ratio declined because the absolute number of CD4 declined, but the CD4:CD8 ratio did not change after administration of dobutamine.

Crary and others found a significant decrease in the CD4:CD8 ratio after administration of adrenaline [5]. These results are in contrast with our findings.

We suggest that the NK cell regulatory effect of dobutamine may be mediated via beta-adrenoceptors, because it is consistent with the findings after administration of adrenaline in other studies.

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TIME COURSE OF NEUROMUSCULAR EFFECTS AND PHARMACOKINETICS OF ROCURONIUM BROMIDE (ORG 9426) DURING ISOFLURANE ANAESTHESIA IN PATIENTS WITH AND WITHOUT RENAL FAILURE

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SUMMARY

We have studied the onset and duration of action and pharmacokinetics of rocuronium bromide (Org 9426) during anaesthesia with nitrous oxide, fentanyl and isoflurane after a single bolus dose of rocuronium 0.6 mg kg^{-1} in nine patients with chronic renal failure requiring regular haemodialysis, and in nine healthy control patients. Blood samples were collected over 390 min and concentrations of rocuronium and its putative metabolites measured using HPLC. Onset time for maximum block, duration of clinical relaxation (T_{125}) and recovery index, were 61 (SD 25.0) s and 65 (16.4) s, 55 (26.9) min and 42 (9.3) min and 28 (12.3) min and 19 (8.8) min, respectively, for patients with and without renal failure. The time for TOF ratio to return spontaneously to 0.7 was 99 (41.1) min and 73 (24.2) min, respectively, in the two groups. None of these differences was significant. The pharmacokinetic data were best described by a three-exponential equation. There were significant differences between patients with and without renal failure in the rates of clearance ($2.5 (1.1) \text{ ml kg}^{-1} \text{ min}^{-1}$ and $3.7 (1.4) \text{ ml kg}^{-1} \text{ min}^{-1}$, respectively) and the mean residence times ($97.1 (48.7) \text{ min}$ and $58.3 (9.6) \text{ min}$) ($P < 0.05$). The differences in other kinetic parameters were not significant. We conclude that the effects of rocuronium may be prolonged in patients with renal disease, because of a decreased clearance of the drug. (Br. J. Anaesth. 1993; 71: 222–226)

KEY WORDS

Neuromuscular relaxants: rocuronium (Org 9426). Pharmacodynamics: renal failure. Pharmacokinetics.

Rocuronium (Org 9426) is a new monoquaternary aminosteroidal neuromuscular blocking drug, with a duration of action similar to that of vecuronium but with a more rapid onset of action [1–3]. Initial animal studies suggested that, as with vecuronium, it is excreted primarily in the bile [4]. However, it became apparent that the pharmacokinetics of vecuronium were affected in the presence of renal disease [5, 6]. In this study we compared the neuromuscular effects and measured the blood concentrations of rocur-

onium after a single bolus dose administered to normal patients and those with renal failure.

PATIENTS AND METHODS

After obtaining written informed consent and Ethics Committee approval, we studied nine patients with normal renal function (ASA I or II) undergoing elective dental or ophthalmic surgery, and nine patients with chronic renal failure requiring regular haemodialysis (ASA III or IV) and undergoing construction of arterio-venous fistulae. Any patient who was receiving medication known to interfere with neuromuscular transmission was excluded, except in the case of patients with renal failure, who were permitted to continue receiving corticosteroids, beta-adrenoceptor antagonists and calcium entry blocking drugs when these were essential medical therapy. Patients who were more than 35 % or less than 20 % of their ideal weight or had abnormal hepatic function were excluded from study. All patients with renal failure had undergone haemodialysis within 2 days before surgery.

After premedication with oral temazepam 20 mg 60 min before operation, anaesthesia was induced with fentanyl $2\text{--}3 \mu\text{g kg}^{-1}$ and thiopentone $3\text{--}5 \text{ mg kg}^{-1}$ and maintained with 66 % nitrous oxide in oxygen and 0.5–1.0 % isoflurane (inspired concentration). Ventilation was assisted to maintain the end-tidal carbon dioxide concentration at 4.5–5.5 %. Additional increments of fentanyl were administered, if required, throughout the procedure.

Heart rate, non-invasive arterial pressure, oxygen saturation and temperature were monitored routinely. Skin temperature over adductor pollicis was kept greater than 32°C throughout the study period by wrapping the arm in cotton wool.

Neuromuscular monitoring was commenced after

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TABLE I. *Physical and biochemical characteristics (mean (SD) [range]) of the two groups. **Significant difference between groups ($P < 0.01$)*

	Renal failure ($n = 9$)		Normal renal function ($n = 9$)
Age (yr)	51 [22–61]		46 [27–64]
Weight (kg)	63 (18.8) [42–91]		69 (13.0) [53–94]
Height (cm)	163 (11.0) [145–182]		164 (12.7) [153–185]
Sex (M/F)	5/4		4/5
Urea (mmol litre ⁻¹)	22.2 (9.6) [14.2–46.0]	**	4.9 (1.1) [3.4–6.4]
Creatinine (mmol litre ⁻¹)	777 (168) [426–940]	**	79 (10.5) [71–95]

induction of anaesthesia using transcutaneous supra-maximal stimuli of 0.2 ms duration to the ulnar nerve at the wrist in a train-of-four (TOF) mode at 2 Hz every 12 s. The resultant force of contraction of adductor pollicis was measured and recorded using a force displacement transducer and neuromuscular function analyser (Myograph 2000, Biometer Ltd). After stabilization of control responses, rocuronium 0.6 mg kg⁻¹ ($2 \times \text{ED}_{95}$ dose) was administered as a fast bolus injection. The times to greater than 5% depression of T1 (lag time) and its maximum depression (onset time) were recorded. Tracheal intubation was performed after the onset of maximum neuromuscular block. The times for spontaneous recovery of T1 to 25, 75 and 90% of control and to a TOF ratio of 0.7 were recorded as measures of the recovery characteristics of this bolus dose.

Anaesthesia was stopped and tracheal extubation performed at the end of surgery. The patients were observed over the next 1 h in the recovery area for any signs of residual muscle weakness.

Venous blood samples (4 ml) were obtained either from a dedicated peripheral cannula or from a central venous catheter before the administration of rocuronium (time zero), and at 1, 3, 5, 10, 15, 30, 60, 90, 150, 210, 270, 330 and 390 min after administration; at 25% recovery of T1 (T1₂₅) and when the TOF ratio was 0.7. The samples were collected in lithium heparinized tubes and centrifuged within 4 h. The plasma was mixed with sodium dihydrogen phosphate buffer solution (0.2 ml to each 1.0 ml of plasma) and stored at -18 °C until required for analysis.

Analysis of rocuronium and its putative metabolites, 17-desacetyl rocuronium and 16 N-desallyl rocuronium was carried out using high pressure liquid chromatography (HPLC) by a method previously described for vecuronium [7] but modified and validated for rocuronium and using 3,17-didesacetyl vecuronium (Org 7402) as the internal standard. After extraction of rocuronium and its putative derivatives from the sample, the compounds were separated by HPLC and the concentrations determined by fluorimetric detection after post-column ion-pair extraction. The precision (reproducibility) of the method is 8% over the range 10–100 000 ng ml⁻¹ for rocuronium, 7% over 10–25 000 ng ml⁻¹ for 17-desacetyl rocuronium and 12% over 10–25 000 ng ml⁻¹ for desallyl rocuronium. The limit of quantification with a precision better than 15% of this HPLC method was 10 ng ml⁻¹ for rocuronium and 20 ng ml⁻¹ for its putative derivatives, from a 1-ml sample of plasma.

Concentration *vs* time data were fitted for individual patients to both two- and three-exponential equations using a computer program for non-linear curve fitting (Multifit, JH Proost, University of Groningen in the Netherlands). This program allows estimation of parameters of various compartmental models using various minimizing algorithms [8]. It uses standard procedures and pharmacokinetic formulae derived from the literature [9]. The method has been validated using datasets and results from other available programs. The appropriate model was determined for each patient by the *F* test. The volume of the central compartment (V_c), steady-state distribution volume (V_{ss}), plasma clearance (Cl), elimination half-life ($T_{1/2}^p$) and mean residence time (MRT) were calculated using standard equations.

Data were subjected to Mann–Whitney *U* test for determination of statistical significance. This test was chosen because of the wide scatter of the results, particularly in patients with renal failure. $P < 0.05$ was considered statistically significant.

RESULTS

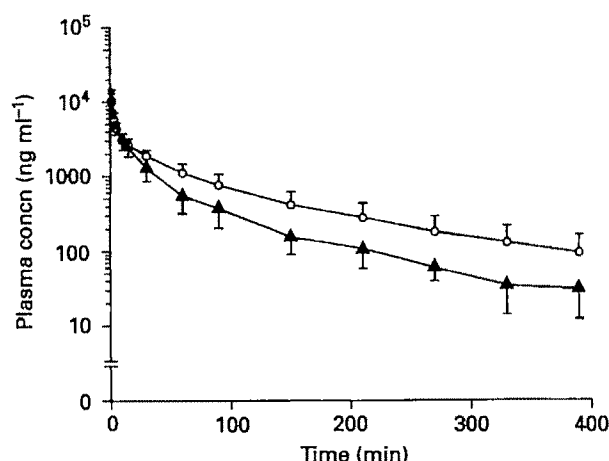
The patients in the two groups were similar in average ages, weights, heights and sex distribution (table I).

The onset and duration of action of rocuronium are given in table II. Neuromuscular block was evident in about 30 s and the onset of maximum block was present in just over 1 min in both groups. The block was complete in all but one patient in the renal failure group and one patient in the control group, in whom the maximum blocks attained were 97% and 96%, respectively. Times to recovery to T1₂₅ were 55 and 42 min, to T1₇₅ 84 and 60 min and to T1₉₀ 101 and 69 min, respectively, in renal failure and normal patients. Times for the TOF ratio to recover to 0.7 were 99 and 73 min, respectively, with recovery indices (times for recovery of T1 from 25% to 75% of control (T1_{25–75})) of 28 and 19 min. Although the times for all these end-points were longer in patients with renal failure, the differences did not attain statistical significance ($P > 0.05$).

Figure 1 shows the mean plasma concentrations of rocuronium over the period of sampling. The data from one patient from each group were excluded: the patient from the control group had received a further increment of rocuronium which interfered with the fitting of the data and the patient from the renal failure group had received metoclopramide which interfered with the analysis of rocuronium. For 14 of 16 patients, the three-exponential equation provided

TABLE II. Characteristics of neuromuscular block (mean (SD) [range]). *Significant difference between groups ($P < 0.05$)

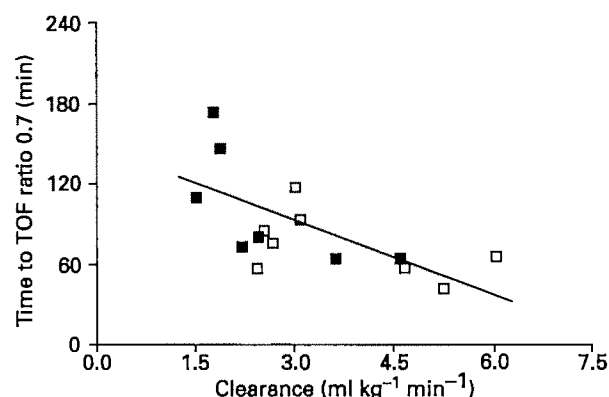
	<i>n</i>	Renal failure patients	<i>n</i>	Normal patients
Lag time (s)	9	29 (7.4) [22–41]	9	31 (6.0) [22–40]
Onset time (s)	9	61 (25.0) [43–124]	9	65 (16.4) [50–98]
Time to recovery (min)				
to $T_{1_{25}}$	9	55 (26.9) [35–115]	9	42 (9.3) [32–60]
to $T_{1_{75}}$	8	84 (36.7) [54–158]	8	60 (16.9) [41–92]
to $T_{1_{90}}$	7	101 (47.4) [64–182]	8	69 (19.5) [47–101]
Time to TOF ratio 0.7 (min)	8	99 (41.1) [62–174]	8	73 (24.2) [40–116]
Recovery index (min)	8	28 (12.3) [12–43]	8	19 (8.8) [10–32]
Plasma concn (ng ml ⁻¹)				
at $T_{1_{25}}$	9	1229 (308) [843–1657]	8	891 (357) [430–1560]
at TOF ratio of 0.7	8	669 (237) [441–1047]	*	8 441 (165) [194–770]

FIG. 1. Plasma concentrations (mean, SD) after administration of rocuronium 0.6 mg kg⁻¹. ○ = Patients with renal failure; ▲ = patients with normal renal function. Significant difference between groups from 15 min onwards ($P < 0.05$).

a significantly better fit compared with a two-exponential equation, and the estimates derived from this were used for evaluation. Data were described by the triexponential equation:

$$C(t) = Pe^{-\alpha t} + Ae^{-\beta t} + Be^{-\gamma t}$$

where C = plasma concentration; t = time interval (min) between administering the bolus dose and collection of the blood sample; P , A and B = the

FIG. 2. Correlation between rates of clearance and time to TOF ratio 0.7 ($r = -0.64$; $P < 0.01$) in patients with normal renal function (□) and patients with renal failure (■).

intercepts; π and α = slopes representing the rate constants of the rapid and slow distribution, respectively; β = slope representing the rate constant of the elimination phase. The intercompartmental rate constants are given in table III.

The mean pharmacokinetic data for each group are given in table IV. V_c (51.0 ml kg⁻¹ and 38.5 ml kg⁻¹) in the renal failure and normal patients, respectively, V^{ss} (211.7 ml kg⁻¹ and 207.1 ml kg⁻¹) and $T_{1/2}^{\alpha}$ (104.4 min and 97.2 min) did not differ significantly between the groups. MRT (97.1 min and 58.3 min) was significantly longer and the Cl (2.5

TABLE III. Intercompartmental rate constants (mean (SD) [range]). *Significant difference between groups ($P < 0.05$)

	Renal failure (<i>n</i> = 8)		Normal (<i>n</i> = 8)
k_{10} (min ⁻¹)	0.062 (0.032) [0.0158–0.1046]	*	0.119 (0.064) [0.0693–0.2545]
k_{12} (min ⁻¹)	0.311 (0.183) [0.1191–0.5739]		0.259 (0.155) [0.0304–0.4946]
k_{21} (min ⁻¹)	0.176 (0.098) [0.1041–0.3784]		0.163 (0.069) [0.0460–0.2797]
k_{13} (min ⁻¹)	0.031 (0.026) [0.0088–0.0768]		0.060 (0.056) [0.0071–0.0896]
k_{31} (min ⁻¹)	0.014 (0.005) [0.0063–0.0207]		0.012 (0.005) [0.0054–0.0177]

TABLE IV. Main pharmacokinetic variables (mean (SD) [range]). V_c = Volume of the central compartment; V^{ss} = volume of distribution at steady state; $T_{1/2}^{\alpha}$, $T_{1/2}^{\beta}$ and $T_{1/2}^{\gamma}$ = fast and slow distribution and the elimination half-lives respectively; Cl = clearance; MRT = mean residence time. *Significant difference between groups ($P < 0.05$)

	Renal failure patients (<i>n</i> = 8)		Normal patients (<i>n</i> = 8)
V_c (ml kg ⁻¹)	51.0 (29.2) [17.99–95.78]		38.5 (21.1) [10.54–78.12]
V^{ss} (ml kg ⁻¹)	211.7 (47.3) [163.56–307.89]		207.1 (48.9) [151.12–287.45]
$T_{1/2}^{\alpha}$ (min)	1.6 (0.7) [0.65–2.34]		1.8 (1.5) [0.79–5.27]
$T_{1/2}^{\beta}$ (min)	24.3 (11.1) [9.38–44.29]		14.8 (5.5) [7.99–23.20]
$T_{1/2}^{\gamma}$ (min)	104.4 (41.0) [69.36–183.03]		97.2 (26.4) [67.94–141.72]
Cl (ml kg ⁻¹ min ⁻¹)	2.5 (1.1) [1.52–4.63]	*	3.7 (1.4) [2.45–6.05]
MRT (min)	97.1 (48.7) [44.69–203.18]	*	58.3 (9.6) [38.41–66.17]

ml kg⁻¹ min⁻¹ and 3.7 ml kg⁻¹ min⁻¹) significantly less (both $P < 0.05$) in patients with renal disease. The plasma concentrations of rocuronium at T1₂₅ were 1229 and 891 ng ml⁻¹, and at TOF ratio 0.7 were 669 and 441 ng ml⁻¹ respectively, in patients with and without renal failure (TOF ratio- $P < 0.05$).

There was a significant negative correlation between the rate of clearance and the time to the spontaneous recovery of TOF ratio to 0.7 ($r = -0.64$; $P < 0.01$) (fig. 2).

There were large between-patient differences in both clinical response and kinetic parameters, particularly in patients with renal disease. No metabolites were detected in plasma in patients from either group.

DISCUSSION

We have shown that rocuronium was a rapidly acting agent with an onset time just greater than 1 min after a bolus of 0.6 mg kg⁻¹ in patients with or without renal failure. This is similar to the findings of Szenohradszky and colleagues [10] using the same dose of rocuronium in patients with renal failure and observed by us in a previous study in healthy patients [11]. Onset of neuromuscular block with rocuronium in renal failure appears to be unlike that after other myoneural blockers such as vecuronium, atracurium and tubocurarine, which have been reported by some workers to show a delayed onset of effect compared with healthy patients [12, 13].

The duration of clinical relaxation (T1₂₅) after rocuronium was prolonged (55 min) in patients with renal disease compared with that in patients with normal renal function (42 min). Because of the relatively large degree of variability present, especially among patients with renal failure, these prolonged recovery characteristics failed to attain statistical significance; it is likely that analysis of results from a larger number of patients may have produced significant findings. This was a first study undertaken in a group of ill patients which has suggested that a longer duration of action of rocuronium is likely in them. Szenohradszky and colleagues reported times of 49 min and 47 min in patients with or without renal failure, respectively [10]; the duration of action in both groups of patients in the present study was also longer than those we have observed in a previous study [11]. The difference is likely to reflect the use of isoflurane in the present study, in contrast with the use of balanced or halothane-supplemented anaesthesia in the earlier study [11]. Some prolongation of recovery with rocuronium during isoflurane and enflurane anaesthesia has been reported previously [14]. The duration of clinical relaxation is, however, much more prolonged in patients with marked hepatic dysfunction [15].

Studies on the excretion of rocuronium suggest that the main mechanism is hepatic uptake and biliary excretion [4, 15]. However, the results from the present study suggest a considerable role for renal clearance. The rates of clearance in patients with normal renal function in the present study are similar to those reported by Wierda and colleagues [16]. The reduced rates of clearance in patients with

renal failure are in keeping with findings in cats in which renal pedicle ligation resulted in a smaller rate of clearance [4]. This study in cats suggested that only about 10 % of an administered dose of rocuronium was excreted in urine. However, our findings of an approximately 30–40 % increase in the indices of duration of action of rocuronium in patients with renal failure would suggest a greater dependence upon renal excretion in man. That this is the case is supported by the findings of Wierda's group, who were able to recover 33 % of unchanged rocuronium from urine [16]. Observation of a significant negative correlation between the rate of clearance and the time to spontaneous recovery of TOF ratio to 0.7 would suggest the decreased clearance to be the responsible factor for a somewhat longer duration of action of rocuronium.

The steady-state volume of distribution for rocuronium is smaller (211.7 ml kg⁻¹ and 207.1 ml kg⁻¹, respectively, in patients with and without renal failure) compared with that reported by Bencini and colleagues [13] for vecuronium (471 ml kg⁻¹ and 510 ml kg⁻¹). This may be a result of the greater fat solubility of vecuronium [16]. For rocuronium, which has a greater dependence on hepatic uptake and biliary excretion [4, 15], it is in hepatic dysfunction that both the kinetics and dynamics show the greatest changes. However, there are to date no published studies which have measured the biliary excretion of rocuronium in humans. Absence of any detectable metabolites would indicate minimal metabolism, which is supported by results from animal studies [4].

The plasma concentrations of rocuronium during recovery from neuromuscular block were greater in patients with renal failure, significantly so at the point of recovery of TOF ratio 0.7 (table II). This may indicate altered sensitivity of the receptor in patients with renal failure. However, this remains speculative, as steady-state plasma concentrations at a particular degree of neuromuscular block (such as $C_{P^{50}}$) were not determined in the present study. Because of the very rapid onset of block, it is difficult to obtain sequential samples after a bolus of rocuronium 0.6 mg kg⁻¹. The present study was not, therefore, designed as a pharmacokinetic-pharmacodynamic modelling study which would have yielded such information.

In conclusion, the results from the present study suggest some prolongation of the effect of a single 0.6-mg kg⁻¹ dose of rocuronium in patients with renal failure, and demonstrated that they show greater between-patient variability than patients with normal renal function. This may be even more significant with larger or repeated doses. The results suggest that the drug should be used with caution in patients with significant renal disease, and that monitoring of neuromuscular block in this group of patients is essential.

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PHARMACODYNAMICS OF MIVACURIUM CHLORIDE IN PATIENTS WITH HEPATIC CIRRHOSIS

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SUMMARY

Ten healthy patients and 25 patients with cirrhosis of the liver (10 Child's A, 10 Child's B and 5 Child's C) received a bolus dose of mivacurium chloride $150 \mu\text{g kg}^{-1}$. The electromyographic response was monitored throughout anaesthesia until recovery of the first twitch of the train-of-four (TOF) (T_1/T_0) to at least 85% and the TOF ratio ($T_4:T_1$) to at least 80%. There was no significant difference between the two groups in the onset of neuromuscular block, but recovery was prolonged in the cirrhotic group compared with the healthy patients (respective mean times to recovery of T_1/T_0 : to 5% = 20.2 vs 11.2 min ($P < 0.05$); to 10% = 23.8 vs 13.4 min ($P < 0.005$); to 25% = 28.4 vs 16.6 min ($P < 0.005$); to 50% = 41.1 vs 20.1 min ($P < 0.005$); to 75% = 43.8 vs 24.9 min ($P < 0.005$). Recovery of $T_4:T_1$ to 70% = 48.1 vs 27.4 min ($P < 0.005$)). Recovery was most prolonged in the Child's C patients. Mean plasma cholinesterase activity was less in the cirrhotic compared with the healthy group (mean 582 (SD 254) iu litre⁻¹ vs 1125 (303) iu litre⁻¹) ($P < 0.001$) and there was a significant negative correlation between plasma cholinesterase activity and all the indices of recovery ($P < 0.001$ for all except recovery index ($P < 0.01$)). We conclude that patients with hepatic cirrhosis may be sensitive to mivacurium, which could be explained, at least in part, by the lesser plasma cholinesterase activity. (Br. J. Anaesth. 1993; 71: 227-231)

KEY WORDS

Neuromuscular relaxants: mivacurium. Pharmacodynamics: liver disease.

The new, short-acting, non-depolarizing neuromuscular blocking agent, mivacurium chloride, is metabolized by plasma cholinesterase. Early studies *in vitro* suggested that this occurs at a rate of 70-88% that of suxamethonium [1, 2]. It is known that the activity of this enzyme is reduced in liver disease [3]. We decided, therefore, to examine the pharmacodynamics of mivacurium in patients with hepatic cirrhosis in comparison with a control group of healthy patients.

An early study of mivacurium failed to demonstrate a consistent relationship between plasma cholinesterase activity and duration of neuromuscu-

lar block in healthy patients, but in these subjects there was little variation in enzyme activity [1]. More recently, a significant negative correlation has been reported in three studies. Phillips and Hunter reported a negative correlation between plasma cholinesterase activity and recovery of T_1/T_0 to 5% after a bolus of mivacurium $150 \mu\text{g kg}^{-1}$ in a study comparing renal failure patients, in whom plasma cholinesterase activity was reduced, and healthy patients [4]. Ostergaard and colleagues also showed a negative correlation between plasma cholinesterase activity and time to return of the first twitch following a bolus dose of $200 \mu\text{g kg}^{-1}$, although they could not demonstrate a correlation when a dose of $100 \mu\text{g kg}^{-1}$ was used [5]. Ali and colleagues showed a negative correlation between plasma cholinesterase activity and the infusion rate of mivacurium required to maintain 90-99% depression of T_1/T_0 in healthy patients [6]. Therefore we also have attempted to correlate pharmacodynamic data with plasma cholinesterase activity.

PATIENTS AND METHODS

The study was approved by the Committee of Safety of Medicines and the Hospital Ethics Committee. All patients gave written, informed consent. We studied 25 patients with hepatic cirrhosis and 10 healthy patients undergoing intermediate, elective surgical procedures requiring neuromuscular block.

All but one of the cirrhotic group were having injection sclerotherapy of oesophageal varices. The remaining patient in that group, and all the healthy patients, were undergoing general surgical or gynaecological procedures, such as inguinal hernia repair or laparoscopy. Hepatic cirrhosis is a chronic disease with a variable degree of functional impairment. Using Child's criteria [7], the cirrhotic group were classified further into three subgroups (A, B and C) in order to examine the influence of the severity of hepatic disease on the pharmacodynamic data. Patients were excluded if they had a clinical history of a neuromuscular disorder, asthma, renal failure, difficult tracheal intubation or were receiving drugs

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TABLE I. *Child's classification for assessing the severity of liver dysfunction* [7]

	Group A	Group B	Group C
Serum bilirubin ($\mu\text{mol litre}^{-1}$)	< 34	34–50	> 50
Serum albumin (g litre^{-1})	> 35	30–35	< 30
Ascites	None	Easily controlled	Poorly controlled
Neurological disorder	None	Minimal	Advanced
Nutrition	Excellent	Good	Poor

known to interfere with neuromuscular transmission or hepatic function. All patients with cirrhosis were receiving oral ranitidine 150 mg twice daily and vitamin K 10 mg daily. Child's B and C patients were also receiving oral spironolactone 100–400 mg daily.

All the patients had preoperative measurements of haemoglobin concentration, platelet count, urea, electrolytes and plasma albumin; liver function tests were also performed. Before operation, plasma cholinesterase activity was measured using a spectrophotometric method [8] and the dibucaine and fluoride numbers were determined [9]. The cirrhotic patients also had prothrombin time and activated partial thromboplastin time measured. Each cirrhotic patient was assigned to the appropriate Child's subgroup (table I). ASA II and III patients had a preoperative chest x-ray and electrocardiogram.

Premedication was with either diazepam 10 mg orally, Cyclimorph (morphine sulphate 10 mg ml^{-1} and cyclizine 50 mg ml^{-1}) 1 ml i.m. or no premedication (in the case of the Child's C cirrhotic patients). Anaesthesia was induced with fentanyl 1 $\mu\text{g kg}^{-1}$ and thiopentone 3–5 mg kg^{-1} , followed by a period of at least 15 min during which the patient breathed isoflurane and 70% nitrous oxide in oxygen, until a stable end-tidal isoflurane concentration of 0.5% (measured using a Capnomac (Datex)) had been achieved. End-tidal carbon dioxide partial pressure was maintained in the range 4.5–5.5 kPa, with positive pressure ventilation when needed, using the Capnomac. During this period, electromyographic monitoring was commenced, applying train-of-four (TOF) stimuli to the ulnar nerve at the wrist at 12-s intervals and recording the surface compound action potential over adductor pollicis using a Medelec MS6 [10]. The arm was wrapped in cotton wool padding, but skin temperature was not measured. When the end-tidal isoflurane concentration was stable, a bolus dose of mivacurium 150 $\mu\text{g kg}^{-1}$ was given into a fast flowing peripheral infusion in the other forearm. Electromyographic monitoring was continued throughout surgery and until the first twitch (T1/T0) had recovered to at least 85% and the TOF ratio (T4:T1) had reached at least 80%. No supplementary doses of mivacurium were given and no anticholinesterase was used. Increments of fentanyl, thiopentone, or both, were given when clinically indicated.

Controlled ventilation was undertaken and tracheal intubation attempted 2 min after admin-

istration of mivacurium. Intubation was graded as excellent (no movement, vocal cords abducted), good (cords abducted, some diaphragmatic movement), poor (intubation achieved but cord and diaphragmatic movement present) or impossible [11]. If intubation was impossible at 2 min, another attempt was made 1 min later.

After injection of mivacurium, evidence of cutaneous histamine release was sought at the injection site and over the face and trunk. Heart rate and indirect arterial pressure were recorded, using a Cardiacap (Datex), before induction, during anaesthesia immediately before the administration of mivacurium, and at 1-min intervals for a period of 5 min after the bolus of mivacurium and thereafter at 5-min intervals. After completion of surgery, and when T4:T1 was greater than 80%, anaesthesia was discontinued and, when ventilation was considered adequate, the trachea was extubated. All patients were assessed in the recovery room for signs of residual curarization by their ability to maintain 5-s head-lift, tongue protrusion and cough [12]. The patients were seen also in the ward the next day to ensure that all cardiovascular variables had remained stable. They were asked specifically about muscle pain and weakness.

Data analysis

The electromyographic recording was analysed to determine the times to 95% and maximum depression of T1/T0 and the maximum depression of T1/T0 achieved. The times to recovery of T1/T0 to 5%, 10%, 25%, 50% and 75%, the recovery index (time for T1/T0 to recover from 25% to 75%), and the time for T4:T1 to return to 70% were also noted.

The onset and recovery data for the cirrhotic and the control groups were compared using the Mann-Whitney *U* test, as was the mean plasma albumin concentration in each group. Plasma cholinesterase activity was correlated with both onset and recovery data using Pearson's correlation coefficient.

RESULTS

The age, weight, sex, plasma cholinesterase activity and plasma albumin concentration of the patients are shown in table II. The patients in the control group were younger than those in the cirrhotic group and there was a slightly greater proportion of female patients in the cirrhotic group.

The measured plasma cholinesterase activity was significantly less in the cirrhotic group (table II). Plasma cholinesterase activity was particularly reduced in the Child's C subgroup (mean 275 iu litre^{-1} , range 200–384 iu litre^{-1}). One patient (Child's C) was found to be heterozygous for the dibucaine resistant gene (dibucaine number 68; normal range 77–83). This patient had a plasma cholinesterase activity of 236 iu litre^{-1} and a fluoride number of 55 (normal range 55–65). All the other patients had normal dibucaine and fluoride numbers.

Serum albumin concentration was significantly reduced in each cirrhotic subgroup compared with values in healthy patients (table II).

TABLE II. Patient characteristics, plasma cholinesterase activity and plasma albumin concentration (mean (SD) [range]) in the two groups and with Child's subgroups shown. * $P < 0.05$; ** $P < 0.001$ compared with healthy group

	Healthy ($n = 10$)	Cirrhotic ($n = 25$)	Child's A ($n = 10$)	Child's B ($n = 10$)	Child's C ($n = 5$)
Age (yr)	42.1 [27–59]	52.8 [32–67]	49.7 [32–60]	53.0 [43–60]	58.4 [48–67]
Weight (kg)	70.1 (11.0) [53–84]	65.7 (10.7) [48–87]	60.6 (6.2) [50–68]	71.3 (14.0) [48–85]	64.6 (3.7) [60–70]
Sex (M:F)	5:5	10:15	4:6	4:6	2:3
Plasma cholinesterase activity (iu litre ⁻¹)	1125 (303) [574–1511]	582 (254)** [200–1432]	687 (282)* [448–1432]	595 (178)** [376–872]	275 (78)** [200–384]
Albumin (g litre ⁻¹)	46.5 (2.6) [43–50]	34.6 (5.9)** [24–46]	37.3 (5.6)** [24–44]	34.2 (5.5)** [29–46]	30 (4.7)** [24–36]

TABLE III. Pharmacodynamic onset data for the two groups and with Child's subgroups shown (mean (SD) [range]). $P < 0.05$; *compared with healthy patients; †compared with Child's C patients

	Healthy ($n = 10$)	Cirrhotics ($n = 25$)	Child's A ($n = 10$)	Child's B ($n = 10$)	Child's C ($n = 5$)
Time to 95 % depression (min)	2.8 (0.9) [1.9–5.1]	2.3 (0.9) [1.4–5.4]	2.2 (0.6) [1.4–3.5]	2.2 (0.6) [1.6–3.3] ($n = 9$)	3.0 (1.6) [1.7–5.4] ($n = 4$)
Time to max. depression (min)	5.2 (1.4) [4.1–8.5]	6.1 (3.9) [2.9–23.0]	4.7 (1.0)† [2.9–6.7]	5.3 (1.5)† [3.9–7.6]	10.7 (7.4)* [4.2–23.0]
Maximum depression (%)	98.7 (1.3) [95.4–99.9]	98.1 (4.0) [80.3–100]	99.1 (0.6) [98.3–99.8]	97.1 (6.3) [80.3–99.8]	97.3 (3.4) [91.5–100]

TABLE IV. Pharmacodynamic recovery data for the two groups and with Child's subgroups shown (mean (SD) [range]). * $P < 0.05$; ** $P < 0.005$ compared with healthy patients; †† $P < 0.01$ Child's C compared with Child's B

Times to (min)	Healthy ($n = 10$)	Cirrhotics ($n = 25$)	Child's A ($n = 10$)	Child's B ($n = 10$)	Child's C ($n = 5$)
T1/T0 = 5 %	11.2 (3.1) [7.7–18.0]	20.2 (8.1)* [7.2–44.5]	17.0 (6.7) [7.2–26.7]	19.2 (4.9)* [11.2–26.2]	30.7 (10.1)**†† [21.6–44.5]
T1/T0 = 10 %	13.4 (3.0) [10.0–19.2]	23.8 (8.9)* [8.5–50.3]	19.6 (7.2)* [8.5–31.1]	22.3 (5.1)** [13.2–29.1]	34.9 (9.1)**†† [26.2–50.3]
T1/T0 = 25 %	16.6 (3.3) [13.0–22.3]	28.4 (10.4)** [11.0–57.0]	23.5 (8.2)* [11.0–37.5]	26.1 (6.3)** [16.0–34.3]	42.9 (8.9)**†† [35.0–57.0]
T1/T0 = 50 %	20.1 (3.9) [15.6–25.8]	34.9 (14.2)** [12.6–81.3]	27.9 (10.1)* [12.6–46.4]	32.0 (7.0)** [19.1–40.8]	54.6 (15.2)**†† [43.0–81.3]
T1/T0 = 75 %	24.9 (6.5) [19.0–40.4]	43.8 (20.8)** [14.6–114.5]	33.5 (13.0)* [14.6–60.2]	40.7 (13.0)** [22.4–68.7]	70.7 (25.4)**†† [52.2–114.5]
T4:T1 = 70 %	27.4 (4.8) [19.8–33.5]	48.1 (20.5)** [19.5–120.7]	39.1 (15.4)* [19.5–70.4]	43.5 (8.5)** [27.6–55.1]	75.0 (25.8)**†† [60.0–120.7]
25–75 % T1/T0	8.3 (4.1) [5.4–19.6]	15.4 (12.8) [3.6–57.5]	9.9 (5.2) [3.6–22.7]	14.6 (12.8) [6.3–50.0]	27.8 (17.1)**†† [15.9–57.5]

There were no significant differences between the onset data in the two groups, although the Child's C patients had a significantly prolonged time to reach maximum depression of T1/T0 (10.7 min) compared with the other Child's subgroups and the healthy group ($P < 0.05$) (table III). Two cirrhotic patients showed resistance to the action of mivacurium: in one Child's B patient, the maximum block achieved was 80.3 % at 23 min, and in one Child's C patient it was 91.5 % at 7.5 min. There was no correlation between onset variables and plasma cholinesterase activity.

In contrast, the recovery from neuromuscular block was significantly prolonged in the cirrhotic group for all variables except the recovery index which, although longer in the cirrhotic group, was not statistically significant (table IV). Even the Child's A subgroup with only minimal hepatic dysfunction had a prolonged recovery from block, of

about 50 % greater than the healthy group. The Child's C subgroup had a markedly prolonged recovery—150 % greater than healthy patients and significantly longer even than the Child's B subgroup (table IV).

In contrast with the onset data, there was a significant negative correlation between all recovery variables and plasma cholinesterase activity (5 % $r = -0.71$; 10 % $r = -0.74$; 25 % $r = -0.79$; 50 % $r = -0.71$; 75 % $r = -0.63$; 90 % $r = -0.62$; 95 % $r = -0.71$; recovery index $r = -0.41$ ($P < 0.001$ for all except recovery index— $P < 0.01$)). This is demonstrated for the time to 25 % recovery of T1/T0 in figure 1. The final recorded height of T1/T0 was greater than 95 % in 15 patients, 90–95 % in 14 patients and 85–90 % in six patients.

Mean arterial pressure decreased after induction of anaesthesia in both groups, with a further small decrease after administration of mivacurium (table

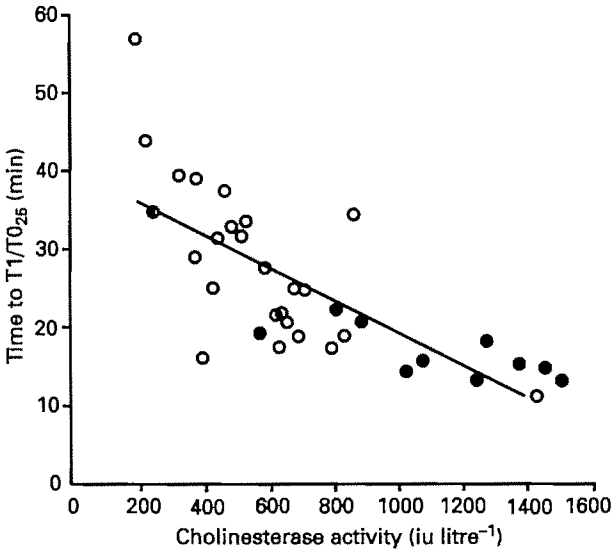


FIG. 1. Time to 25% recovery of T1/T0 ($T_1/T_{0.25}$) plotted against cholinesterase activity. Pearson's correlation coefficient: $r = -0.79$; $P < 0.0001$. ● = Healthy patients (controls); ○ = cirrhotic patients; ◐ = heterozygous patient.

V). Tracheal intubation was performed after the 2 min recording. Mean arterial pressure and heart rate increased after intubation.

Cutaneous histamine release was noted in five patients, two in the control group (20%) and three in the cirrhotic group (12%). This was not associated with any significant cardiovascular change.

In seven (70%) of the healthy patients and 19 (76%) of the cirrhotic patients, intubation conditions were excellent or good at 2 min. Conditions were poor at this time in one (10%) healthy and five (20%) cirrhotic patients and impossible in two (20%) healthy and one (4%) cirrhotic patient. In two of this latter group, intubation was impossible until 5 min, because of clinically inadequate neuromuscular block.

There was no indication of recurarization in the recovery room after anaesthesia. There were no complaints of muscle pains or weakness in the first 24 h after operation. All patients were satisfied with their anaesthetic.

DISCUSSION

The main finding of the present study was the increased duration of action of mivacurium in patients with hepatic cirrhosis. In healthy patients, mivacurium has a shorter duration of action than the

older non-depolarizing agents: the time to 10% recovery of T1/T0 of 13 min contrasts with 27 min for vecuronium and 39 min for atracurium [13]. This difference was reduced in patients with mild to moderate cirrhosis, in whom the time to 10% recovery of T1/T0 of 20.8 min is little less than the times for vecuronium (23 min) or for atracurium (29 min) [14]. This therefore negates the main beneficial property of mivacurium in this group of patients. In severe cirrhosis (Child's group C), although the number of such patients in the present study was small, the duration of mivacurium appeared to be so prolonged and so unpredictable as to vitiate its use.

Atracurium is currently the drug of choice in cirrhotic patients with severe liver dysfunction (Child's group C), because of its predictable duration of action, which is unrelated to the degree of hepatic dysfunction [14]. In these patients atracurium is clearly superior to mivacurium. This finding is supported by early reports of the use of mivacurium in patients with end-stage hepatic failure undergoing liver transplantation, in whom the duration of action was found to be three times greater than in healthy patients (25% recovery T1/T0: 57 min *vs* 19 min) [15]. Indeed, atracurium would seem to be the only non-depolarizing neuromuscular blocking agent in common use to have a duration of action independent of liver function. All the steroidal agents have been shown to have prolonged elimination or effect in end-stage liver disease [16–18].

The prolonged effect of mivacurium in patients with hepatic disease can probably be explained by the reduced plasma cholinesterase activity, which has long been recognized to occur in such patients [3]. It is unlikely that the differences between the groups in age or the slight difference in sex ratio would account for such a marked effect. This study has shown a significant negative correlation between several indices of recovery and the patient's pre-operative plasma cholinesterase activity. Such findings have been reported in patients with chronic renal failure given mivacurium [4], but not in an earlier study of the drug given to healthy subjects [1]. This may result from a less variable plasma cholinesterase activity in healthy subjects than in a population with renal disease and, consequently, any relationship would be more difficult to detect. There are also differences in the methods used to assay plasma cholinesterase. Although plasma cholinesterase activity needs to be very markedly reduced before the duration of action of suxamethonium is

TABLE V. Cardiovascular variables before the bolus dose of mivacurium and percentage changes after mivacurium compared with post-induction values (mean [range]). Tracheal intubation was attempted after the 2-min recording

Time	MAP (mm Hg)		Heart rate (beat min ⁻¹)	
	Healthy	Cirrhotic	Healthy	Cirrhotic
Before induction	99.6 [90–108]	97.8 [77–115]	83.2 [64–110]	80.6 [59–107]
After induction	82.3 [65–111]	86.6 [70–113]	70.6 [60–85]	78.0 [50–120]
After mivacurium (% change)				
1 min	–1.6 [–12 to +13]	–6.6 [–30 to +42]	–6.0 [–23 to +10]	–4.4 [–24 to +13]
2 min	–3.2 [–15 to +31]	–8.0 [–30 to +47]	–3.3 [–23 to +24]	–2.0 [–28 to +58]

prolonged [19], it is not known if this is true also for mivacurium. This study would suggest, however, that potentiation of neuromuscular block produced by mivacurium may occur even if the plasma cholinesterase activity is only slightly reduced.

The onset of action of mivacurium in patients with hepatic cirrhosis compared with normal control subjects is also of interest. There was a tendency in this study for onset of block, as measured by time to 95% depression, to be more rapid in the Child's A and B patients, although this was not statistically significant (table III). Patients with hepatic cirrhosis have altered haemodynamics, in particular increased cardiac output [20], which may enhance delivery of the drug to the site of action [21] and hasten the onset of block. This explanation is less plausible, however, in Child's group A patients, in whom cardiac output and speed of circulation are not likely to be substantially increased.

In contrast, two patients with severe liver dysfunction showed considerable resistance to the action of mivacurium, similar to that reported by Dundee and Gray for tubocurarine [22]. It may be that the increased volume of extracellular fluid known to occur in severe cirrhosis could have led to an increase in the volume of distribution of mivacurium, as occurs with pancuronium, fazadinium and atracurium, and hence to a less intense block [16, 23, 24]. Pharmacokinetic studies of mivacurium are required to establish the relationship between volume of distribution and effect.

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PHARMACODYNAMICS OF MIVACURIUM IN CHILDREN, USING A COMPUTER-CONTROLLED INFUSION

O. A. MERETOJA AND K. T. OLKKOLA

SUMMARY

A computerized infusion system was used to determine requirement for a mivacurium infusion to maintain a 95% and a 50% neuromuscular block in 21 children aged 1–15 yr. Neuromuscular block was measured by adductor pollicis EMG and anaesthesia maintained with 66% nitrous oxide in oxygen and alfentanil 50–100 $\mu\text{g kg}^{-1} \text{h}^{-1}$. The targeted neuromuscular block was reached within mean 5 (SD 3) min from initiation of an infusion. Mivacurium requirement for 95% neuromuscular block was 950 (350) $\mu\text{g kg}^{-1} \text{h}^{-1}$, which represented an hourly requirement of 6.8 (1.6) individual ED_{95} doses. Infusion requirement for 50% neuromuscular block averaged 350 (150) $\mu\text{g kg}^{-1} \text{h}^{-1}$. There was a significant negative correlation between infusion rate and age of a patient. Great individual variation of the infusion rate makes a computerized infusion an easy method to achieve and maintain a desired level of neuromuscular block. No side effects of mivacurium were noticed. (Br. J. Anaesth. 1993; 71: 232–237)

KEY WORDS

Anaesthesia: paediatric. Equipment: computers. Neuromuscular relaxants: mivacurium.

Mivacurium, a short-acting, non-depolarizing neuromuscular blocking agent, has been studied in children, to create dose-response curves [1, 2] and to maintain 89–99% neuromuscular block with a continuous infusion [3, 4]. A common finding was that the requirement of mivacurium was greater in children than in adults under comparable study circumstances. If mivacurium is administered as incremental bolus doses, children would require a dose of 0.1 mg kg^{-1} approximately once every 6–7 min during maintenance of anaesthesia [3, 4]. However, individual titration of a maintenance dose would be necessary, as the between-patient variability of infusion requirement is three- to four-fold during general anaesthesia [3, 4]. Consequently, it would be practical to have an automatic infusion controller to maintain a desired neuromuscular block. Our aim was to study the pharmacodynamics of mivacurium in children using a closed-loop feedback control system to determine mivacurium requirements to maintain two different levels of

neuromuscular block (95% and 50%) during nitrous oxide-oxygen-alfentanil anaesthesia in children.

PATIENTS AND METHODS

After approval by the Institutional Ethics Committee and after parental informed consent, we studied 21 children aged 1–15 yr, ASA I or II, undergoing elective abdominal or orthopaedic surgical procedure. The duration of surgery was estimated to exceed 90 min. Patients were excluded if they had renal or hepatic disease or were receiving any medication known to affect neuromuscular transmission.

Patients received oral midazolam 0.4 mg kg^{-1} (maximum dose 15 mg) 45 min before anaesthesia, as premedication, if needed. Anaesthesia was induced with alfentanil 50–100 $\mu\text{g kg}^{-1}$ and thiopentone 4–6 mg kg^{-1} , and maintained with an infusion of alfentanil 50–100 $\mu\text{g kg}^{-1} \text{h}^{-1}$ and 66% nitrous oxide in oxygen. ECG, SpO_2 , non-invasive arterial pressure and palmar skin temperature (Cardiicap, Datex, Helsinki, Finland), were monitored during anaesthesia. Ventilation was controlled to maintain end-tidal carbon dioxide partial pressure in the range 5.0–5.5 kPa.

Neuromuscular transmission was monitored using an electromyograph (Relaxograph, Datex). Surface electrodes were attached over the ulnar nerve at the wrist, and over adductor pollicis and the volar surface of the base of the forefinger to obtain an optimal EMG signal [5]. Train-of-four (TOF) stimulation was applied every 20 s and calibration of the neuromuscular monitoring device was carried out 5–10 min after induction of anaesthesia. Thereafter, a stable baseline calibration signal was awaited before administration of mivacurium. Mivacurium was used in a concentration of 2 mg ml^{-1} , or diluted 1:1 with physiological saline if the patient's body weight was less than 15 kg.

Each patient received a bolus dose of mivacurium 0.1 mg kg^{-1} and maximal neuromuscular effect following this dose was recorded. Thereafter, a second identical bolus dose was administered and the trachea

TABLE I. Patient characteristics, maximal neuromuscular block (NMB) produced by mivacurium 0.1 mg kg⁻¹ and individual ED₉₅ dose and infusion requirements of mivacurium in children. Infusion requirement to maintain 95% NMB is calculated also as ED₉₅ doses per hour (infusion rate/ED₉₅ dose)

Age (yr)	BW (kg)	MaxNMB (%)	ED ₉₅ (µg kg ⁻¹)	Infusion requirement for		
				50% NMB (µg kg ⁻¹ h ⁻¹)	95% NMB (µg kg ⁻¹ h ⁻¹)	95% NMB (ED ₉₅ h ⁻¹)
1.2	10.0	67	153	458	955	6.2
1.4	11.5	75	141	390	880	6.2
1.7	9.7	90	115	338	975	8.6
1.7	12.2	19	244	843	2131	8.8
2.1	9.3	71	148	392	877	5.9
2.4	12.3	85	125	293	976	7.8
2.9	13.4	76	140	361	1112	7.9
4.3	16.0	66	154	552	1229	8.0
6.6	20.0	95	100	179	457	4.6
7.1	20.8	72	145	226	676	4.7
7.3	37.5	57	168	452	888	5.3
7.8	29.2	53	174	344	1263	7.3
8.7	31.0	72	146	294	1217	8.3
9.1	45.8	88	118	329	983	8.3
9.8	39.0	97	91	315	650	7.1
10.5	33.4	94	103	318	881	8.6
10.7	53.0	98	89	232	760	8.5
10.8	39.3	77	138	377	950	6.9
12.4	32.0	59	166	172	777	4.7
14.5	62.5	84	126	183	481	3.8
15.2	72.0	75	141	360	879	6.2
Mean	7.1	29.0	75	353	952	6.8
SD	18.3	18	34	148	346	1.6

intubated when neuromuscular block (depression of the first EMG response in the TOF series of responses) remained greater than 90%. Thereafter, a closed-loop, computer-controlled infusion of mivacurium was commenced using a Fresenius injectomat cp-IS pump (Fresenius AG, Bad Homburg, Germany) [6, 7] (see Appendix), and the target neuromuscular block was set to 95%. Infusion was initiated based on the response to the first bolus doses of mivacurium. When at least 40 min of infusion time had elapsed, the target neuromuscular block was re-set to 50% and a second 40 min of infusion time was recorded. At the end of anaesthesia, residual neuromuscular block was allowed to recover spontaneously before removal of the tracheal tube.

Average infusion requirements of mivacurium to maintain a 95% and a 50% neuromuscular block and the performance of the controller were derived from computer recordings (see Appendix). These infusion requirements were calculated on a body weight basis (in µg kg⁻¹ h⁻¹). In addition, we determined the number of individual ED₉₅ doses of mivacurium that would be required hourly to maintain a 95% neuromuscular block. For this purpose, an estimate of an individual ED₉₅ dose was calculated on the basis of the maximal neuromuscular response after the initial 0.1-mg kg⁻¹ doses of mivacurium, using the method described earlier [8]: a point which represented the 0.1-mg kg⁻¹ dose and the maximal neuromuscular effect produced by this dose was plotted on a log-probit paper on which parallel lines with a slope of 6.5 probit log⁻¹ were drawn. This slope is an average of those determined for mivacurium in children [1, 2], and reflects that of an earlier study of atracurium [8]. An estimated individual ED₉₅ dose of mivacurium was determined by analysing the dose required to produce a 95%

neuromuscular block when a line with a slope of 6.5 probit log⁻¹ was drawn via the one dose-response point determined for that individual child [8].

For statistical purposes, linear regression analysis and Student's *t* test for paired data were used, when appropriate. *P* < 0.05 was regarded as significant. All values are presented as mean (SD).

RESULTS

Patient characteristics are shown in table I. Palmar skin temperature was greater than 34 °C at all times in every child, mean temperature being 35.2 (0.7) °C. The initial 0.1-mg kg⁻¹ dose of mivacurium did not produce any systematic changes in arterial pressure or heart rate: average arterial pressure and heart rate before administration of mivacurium were 90/46 mm Hg (mean 63 mm Hg) and 84 beat min⁻¹, respectively. Systolic, diastolic and mean arterial pressures increased and heart rate decreased by 4 (6), 0 (7) and 3 (6) mm Hg, and by 2 (4) beat min⁻¹, respectively, within 3 min after the administration of mivacurium (not significant).

The maximal effect after mivacurium 0.1 mg kg⁻¹ was attained earlier in younger patients. There appeared to be a linear relationship between age and onset time (time from administration to maximal effect) of mivacurium (fig. 1). Based on linear regression analysis, onset time increased by 1.4 (0.4) min for every 10 yr of age (*P* = 0.0024). Average maximum neuromuscular block after mivacurium 0.1 mg kg⁻¹ was 75 (18)% (range 19–98% block) (table I). The mean individual ED₉₅ dose of mivacurium calculated from these maximal responses was 139 (34) µg kg⁻¹ (table I).

The computer maintained neuromuscular block within a range of 85–99% after 5.5 (5) min from

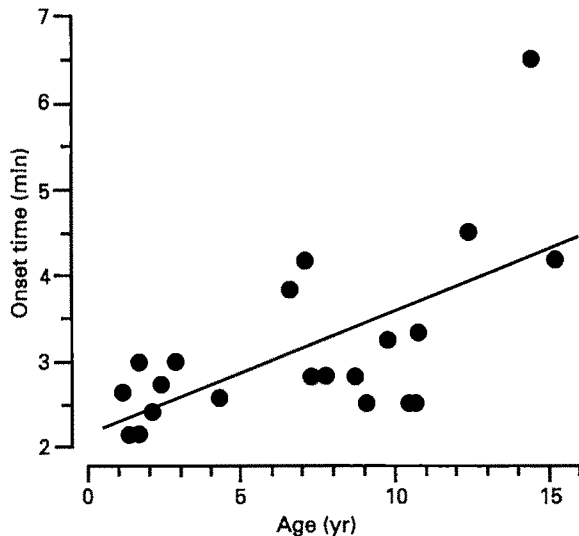


FIG. 1. Correlation between onset time of mivacurium 0.1 mg kg^{-1} and age. Onset time = $0.14 \times \text{age} + 2.2 \text{ min}$; $r = 0.626$; $P = 0.0024$.

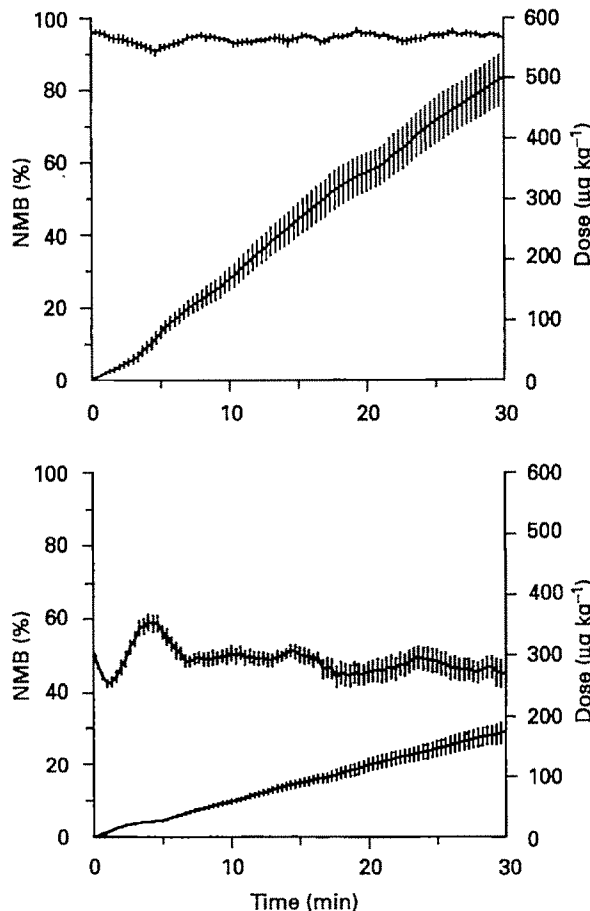


FIG. 2. Time-course of targeted 95% (upper graph) and 50% (lower graph) neuromuscular blocks (NMB) (upper curves) and the cumulative infusion requirement (dose) of mivacurium (lower curves) (mean, SEM). Note that the targeted neuromuscular block was maintained relatively stable within a few minutes of the block first passing the target value.

commencement of the computer-controlled infusion of mivacurium. When the target neuromuscular block was set to 95% or 50%, the average neuromuscular blocks were 94.5 (0.4)% or 49.5 (1.3)%, and mean (SD) from target 3.4 (1.2)% or 8.1 (2.7)%,

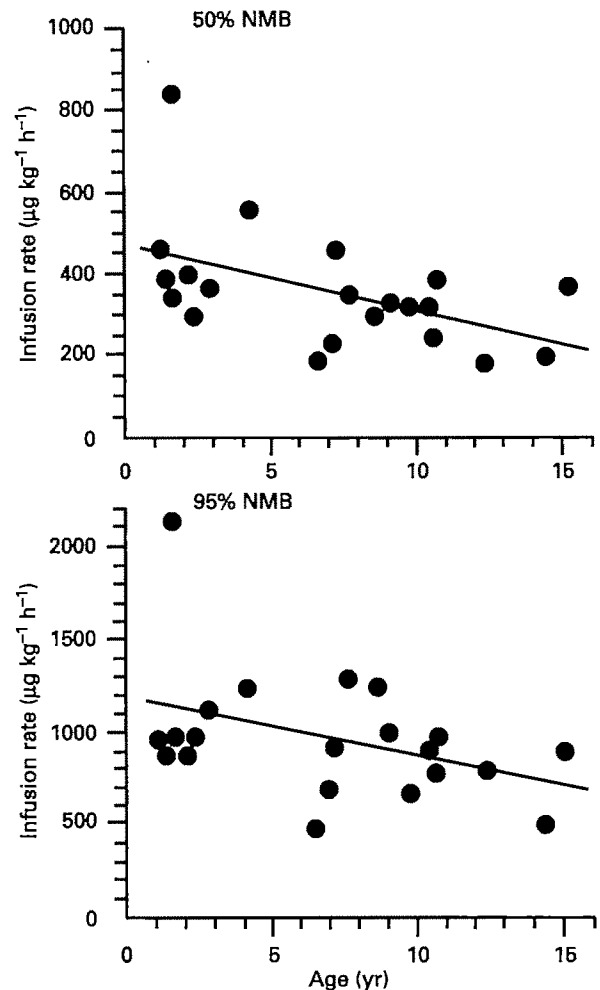


FIG. 3. Correlation between age of child and infusion requirement for mivacurium to maintain a 50% (upper graph) and a 95% (lower graph) neuromuscular block (NMB). The younger the child, the greater the infusion requirement. 50% NMB: $I_{50}^{88} = -17 \times \text{age} + 470 \text{ } \mu\text{g kg}^{-1} \text{ h}^{-1}$; $r = 0.505$; $P = 0.0197$. 95% NMB: $I_{95}^{88} = -33 \times \text{age} + 1180 \text{ } \mu\text{g kg}^{-1} \text{ h}^{-1}$; $r = 0.424$; $P = 0.0553$.

respectively. Mean durations of mivacurium infusion for the two blocks were 46 (12) min and 47 (17) min, respectively. Neuromuscular function recovered from 5% to 50% of the baseline function within 5 (1) min after re-setting of the target block to 50%. After cessation of the computer-controlled infusion at the end of surgery, neuromuscular function recovered from 50 to 100% in 6 (2) min.

Infusion requirement of mivacurium to maintain a 95% neuromuscular block averaged 950 (350) $\mu\text{g kg}^{-1} \text{ h}^{-1}$. This represents an hourly requirement of 6.8 (1.6) individual ED_{95} doses (table I). Infusion requirement of mivacurium to maintain a 50% neuromuscular block was 350 (150) $\mu\text{g kg}^{-1} \text{ h}^{-1}$. Figure 2 shows a time-course of the mean neuromuscular block targeted to 95% and 50%, and simultaneous cumulative infusion requirements of mivacurium. There was a negative correlation between age and infusion requirement of mivacurium to maintain a 50% neuromuscular block: infusion requirement decreases by 170 (70) $\mu\text{g kg}^{-1} \text{ h}^{-1}$ for every 10 yr of age ($P = 0.0197$). Age appeared to correlate also with infusion requirement of mivacurium to maintain a 95% neuromuscular block ($P = 0.0553$) (fig. 3).

DISCUSSION

Previous studies of mivacurium infusion in paediatric patients have used open control of infusion [3, 4] and during the first 15 min of mivacurium infusion, frequent adjustments of rate have been required. In the present study, it took an average of 5 min for the controller to find a relatively steady response at 95 % or at 50 % neuromuscular block.

During nitrous oxide in oxygen and alfentanil anaesthesia, children required an average of mivacurium $950 \mu\text{g kg}^{-1} \text{h}^{-1}$ ($16 \mu\text{g kg}^{-1} \text{min}^{-1}$) to maintain a 95 % neuromuscular block. This rate is similar to that reported earlier for children during open control of mivacurium infusion [3, 4]. It seems, therefore, that use of a computer-controlled infusion device does not confer smaller requirements for mivacurium than a conventional infusion device. Also, the variability of individual infusion requirements (a 40 % coefficient of variation) and the range of individual infusion rates (four- to five-fold differences) were similar to those of earlier studies using a conventional infusion service.

We included a greater age range of patients in our study than have previous paediatric infusion studies (1–15 yr *vs* 2–10 or 2–12 yr [3, 4]). Our finding of a significant correlation between infusion requirement and age is clinically important. All studies have found that children require greater infusion rates of mivacurium than adults [9, 10]; it is not clear why younger children required even greater infusion rates than older children. Mivacurium is degraded by plasma cholinesterase; this enzyme has similar activity in the age range from young infants to adolescents [11], therefore variations in its activity cannot be used to explain the greater maintenance requirement of mivacurium in children. There may, however, be routes of elimination of mivacurium other than via degradation by this enzyme [3]. Plasma clearance of mivacurium, in common with that of atracurium [12], may, indeed, be greater in children than in adults and result in greater maintenance requirements when these are calculated on a body weight basis. However, it is noteworthy that the infusion rate when calculated as $\text{mg m}^{-2} \text{h}^{-1}$ was not related to patient age ($r = 0.088$, $P = 0.704$), and does not differ between children and adults.

We tried to clarify further maintenance requirements of mivacurium in children. To do this, we calculated an hourly infusion requirement for a 95 % neuromuscular block as a fraction of an individual ED_{95} dose (infusion rate/ ED_{95} dose). An hourly infusion requirement of mivacurium for a 95 % neuromuscular block was 6.8 (1.6) individual ED_{95} doses. It is of great interest that a similar value can be derived from adult studies [9, 13]. These results suggest that the greater infusion requirement of mivacurium in childhood is related to a single dose requirement—that is, to the potency of mivacurium in children. When the potency is taken into account, children require amounts of mivacurium per unit time similar to those for adults.

We could not find any correlation between age of child and infusion requirement when the latter was calculated as a fraction of individual ED_{95} dose ($r =$

0.286, $P = 0.208$). Earlier, we found hourly requirements of 0.4–0.6 individual ED_{95} doses to maintain a 95 % neuromuscular block with a long-acting neuromuscular blocking agent (alcuronium or pancuronium [14, 15]) and 1.8–2.0 individual ED_{95} doses for drugs with intermediate duration of action (atracurium or vecuronium [16, 17]). It seems, therefore, that a fractional figure representing the number of individual ED_{95} doses of a particular neuromuscular blocking agent required hourly to maintain a 95 % neuromuscular block can effectively differentiate the duration of action of these agents.

We found a somewhat greater value for ED_{95} doses of mivacurium in our children than those published previously (139 *vs* 103 and 110 $\mu\text{g kg}^{-1}$ [1, 2]). The method that we used to estimate individual ED_{95} doses [8] should be reliable and result in correct dose–response estimates. The most likely explanation for the apparent difference between these doses is the method by which original dose–response calculations were utilized [18]. If the ED_{95} doses of mivacurium in children are recalculated from previous studies [1, 2] using only data from the patient groups who did not have 100 % neuromuscular block, then ED_{95} doses are 130–140 $\mu\text{g kg}^{-1}$. Our results were very close to these values, and indicate that the ED_{95} dose for mivacurium in children is twice that in adults [13].

The mivacurium requirement to maintain a 50 % neuromuscular block has not been evaluated previously. We found a requirement of 350 $\mu\text{g kg}^{-1} \text{h}^{-1}$, which is 37 % of the dose of mivacurium required to maintain a 95 % block. If an infusion rate–response curve is drawn for mivacurium, it has a slope of 3.8 (0.8) probit $\log^{-1}$. This is less than the slope for a single-dose dose–response curve (6.5 probit $\log^{-1}$), but comparable to that for atracurium infusion in adults [19]. Receptor binding characteristics may explain this difference in slopes: during a bolus administration, a competitive neuromuscular blocker may favour block of separate receptors as a consequence of greater affinity to one of the two α -subunits in the neuromuscular receptor [20]. During infusion administration, effects of time constants on receptor binding are minimized and some of the molecules of blocker may be “wasted” by binding to α -units the counterparts of which are already occupied. Thus the ratio $\text{ISS}_{95}:\text{ISS}_{50}$ (ISS = steady-state rate of infusion) is greater than the ratio $\text{ED}_{95}:\text{ED}_{50}$, resulting in a less steep slope for an infusion rate–response curve than for a dose–response curve.

In this study, we found a significant correlation between age and onset time of mivacurium. This finding corroborates previous studies in which onset time of different neuromuscular blocking agents has been evaluated in paediatric patients [21, 22]. Young children circulate their blood volume in less than 30 s, compared with 1 min in older children [23]. This may explain, in part, a shorter onset time in younger children. At the end of anaesthesia, we did not use anticholinesterase agents to antagonize residual neuromuscular block; nevertheless, spontaneous recovery of neuromuscular block was complete in a shorter time than in adults [13]. This

reflects the short recovery index and greater spontaneous recovery rate of the neuromuscular function after use of mivacurium [4, 13], and may be related to increased clearance of mivacurium in children compared with adults.

In conclusion, a computer-controlled infusion of mivacurium was easy to use in children aged 1–15 yr. The controller reached the targeted neuromuscular block within 5 min of the start of the infusion. Infusion requirement was greatest and onset time least in younger patients.

APPENDIX

MODEL-DRIVEN COMPUTERIZED INFUSION OF MIVACURIUM

A two-compartment, open mammillary model with a hypothetical effect compartment linked to the central compartment was assumed to represent a valid model for the pharmacokinetics of mivacurium [24, 25]. The integrated pharmacokinetic and pharmacodynamic model we used consists of two formulae (both given as a function of time, t), one representing the relationship between the function for drug input, $I(t)$, and the concentration of the drug in the effect compartment, $C_e(t)$:

$$C_e(t) = \int_0^t dr' G(t-t') I(t') \quad (1)$$

and one representing the relationship between concentration $C_e(t)$ and effect $E(t)$ [26]:

$$E(t) = \frac{E_{\max}[C_e(t)]^\gamma}{C_o^\gamma + [C_e(t)]^\gamma} \quad (2)$$

The function $G(t)$ is given by the effect compartment concentration after bolus administration of a unit dose [27]:

$$G(t) = \left[\frac{A}{k_{eo} - \alpha} e^{-\alpha t} + \frac{B}{k_{eo} - \beta} e^{-\beta t} + \frac{A(\beta - k_{eo}) + B(\alpha - k_{eo})}{(\alpha - k_{eo})(\beta - k_{eo})} e^{-k_{eo} t} \right] k_{eo} \quad (3)$$

where A and B = zero-time intercepts; α and β = exponential disposition rate constants describing the decay of plasma concentrations $C_p(t)$ after bolus administration of a unit dose [$C_p(t) = Ae^{-\alpha t} + Be^{-\beta t}$]; k_{eo} = elimination rate constant for the effect compartment; $E_{\max}$ = maximum effect; C_o = concentration at half-maximal effect; γ = a value describing the steepness of the concentration-response curve.

The initial values used for the variables were as follows: $A = 5.2 \times 10^{-8}$ mg kg⁻¹ litre⁻¹; body weight (kg), $B = 6.8 \times 10^{-4}$ mg kg⁻¹ litre⁻¹; body weight (kg), $\alpha = 0.87$ min⁻¹, $\beta = 0.12$ min⁻¹, $k_{eo} = 0.13$ min⁻¹, $E_{\max} = 100\%$ (T1 0% from control); $C_o = 0.1$ mg litre⁻¹; $\gamma = 3.5$. The initial values were selected on the basis of previous computer simulations.

By applying the superposition principle, it is possible to calculate the concentration of mivacurium in the effect compartment at any moment and during any drug administration scheme. Equations (1) to (3) give a full description of the drug input-effect relationship. Given a target, equation (2) may be solved for the necessary concentration in the effect compartment. The pharmacokinetic model, equation (1), may be used subsequently for the calculation of the drug input function [28].

If the measured neuromuscular block was within 2% of the desired neuromuscular block, an infusion scheme was used to maintain the current effect of mivacurium, as predicted by the pharmacokinetic-dynamic model (formulae (1) and (2)). Otherwise, the difference between the measured and predicted neuromuscular block was used to correct the model variables. The updated values were used to calculate the new infusion scheme for achieving and maintaining the desired neuromuscular block. This cycle was performed every 20 s. Adjustment of the variables of the pharmacokinetic-dynamic model was begun 2 min after activation of the closed-loop system.

ADAPTATION ALGORITHM

It is apparent that equation (2) is scale invariant with respect to the transformation $(C_o, C_e) \rightarrow (\lambda C_o, \lambda C_e)$ for any number $\lambda \neq 0$. Consequently, the insertion of equation (1) to equation (2) does not depend on A , B and C_o , but only on the ratios A/C_o and B/C_o , thus allowing an estimate not of clearance or volume of distribution, but only of the microconstants k_{10} , k_{12} , k_{21} and the amount of drugs in diverse compartments. This is, however, sufficient for determining the drug input function. A complete adaptation would require the estimation of A/C_o , B/C_o , α , β , k_{eo} . We chose to update during the feedback control only the variables A/C_o and B/C_o . This allowed adaptation of the initial bolus to achieve a certain effect (short term control) and the steady state infusion rate to maintain the given effect (long term control).

The effect E may be regarded as a function of A and B and the drug input $I(t)$:

$$E(t) = E(A, B, I(t))$$

Denoting by $A + \delta A$ and $B + \delta B$ the true hybrid constants for an individual subject, the difference between measured and predicted effect (ΔE) can be expanded in a Taylor series, as follows:

$$\begin{aligned} \Delta E &= E(A + \delta A, B + \delta B, I(t)) - E(A, B, I(t)) \\ &= (\partial E / \partial A) \delta A + (\partial E / \partial B) \delta B + \dots \end{aligned} \quad (4)$$

In conjunction with the condition to minimize the expression $\delta A^2 + \delta B^2$, equation (4) was used to solve for δA and δB . A change in the range of $\pm 10\%$ of the previous values was allowed in each update. From the updated values, new microconstants were calculated that served to correct the drug input function.

CONTROLLER PERFORMANCE

Controller performance was measured by calculating the mean offset from target and mean SD from target during feedback infusion. Feedback infusion was said to begin when block returned from overshoot to target of 95% after the initial bolus. The variables describing the controller performance were calculated for two periods: the first lasted from the beginning of the feedback infusion until the target was changed to 50%; the second lasted from the time when neuromuscular block of 50% was reached for the first time after the change of target until the closed-loop control was relinquished at the end of surgery. Both variables were calculated every 20 s. Mean offset was calculated by:

$$\text{Mean offset} = \sum_{i=1}^n (s - b_i) / n$$

where s = set-point (target); b_i = neuromuscular block measured every 20 s during feedback infusion; n = number of measurements.

Mean SD from target was SD for mean offset defined above.

ASYMPTOTIC STEADY-STATE RATE OF INFUSION OF MIVACURIUM

To estimate the asymptotic steady-state rate of infusion of mivacurium (I^{ss}), we used non-linear curve-fitting to fit the following formula to the curve representing the cumulative dose requirement of mivacurium [29]:

$$\text{Cumulative dose of mivacurium} = D(1 - e^{-kt}) + I^{ss}t$$

where D = amount of mivacurium contained in its apparent distribution volume; k = relative rate of distribution of mivacurium; I^{ss} = asymptotic steady-state rate of infusion of mivacurium; t = duration of mivacurium administration.

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EFFECT OF PREOPERATIVE I. M. ADMINISTRATION OF DICLOFENAC ON SUXAMETHONIUM-INDUCED MYALGIA

S. KAHRAMAN, S. ERCAN, U. AYPAR AND K. ERDEM

SUMMARY

We have studied the effects of preoperative administration of diclofenac on suxamethonium-induced myalgia, plasma met-enkephalin-like activity (E-LA), prostaglandin E₂-like activity (PGE₂-LA), leukotriene C₄-like activity (LTC₄-LA) and histamine-like activity (H-LA). Thirty-four ASA I patients undergoing elective ophthalmic surgery were allocated randomly to two groups to receive either saline placebo or diclofenac 75 mg i.m. 20 min before operation, in a double-blind design. Anaesthesia was induced with thiopentone 5–7 mg kg⁻¹ followed by suxamethonium 1.5 mg kg⁻¹ and maintained with 67% nitrous oxide and halothane in oxygen. Plasma PGE₂-LA, LTC₄-LA, H-LA and E-LA were measured before premedication, 1 min after the administration of suxamethonium and 24 h after operation. Muscle fasciculations, intubation conditions and postoperative myalgia were graded numerically. Postoperative myalgia in the diclofenac group was significantly ($P < 0.05$) less (47.1%) than in the control group (76.5%). Post-suxamethonium and 24-h concentrations of plasma PGE₂-LA and LTC₄-LA were also significantly ($P < 0.05$) greater than baseline in the control group. Plasma H-LA was increased in both groups after suxamethonium and this increase was significant ($P < 0.05$) in the control group. We conclude that diclofenac reduces significantly the incidence and intensity of suxamethonium-induced myalgia. (Br. J. Anaesth. 1993; 71: 238–241)

KEY WORDS

Analgesics: diclofenac. Complications: myalgia. Neuromuscular relaxants: suxamethonium.

Several methods have been suggested to prevent suxamethonium-induced muscle pain or to decrease its severity; the most common is pretreatment with a small dose of a non-depolarizing neuromuscular blocking drug before induction of anaesthesia. However, this method is not free of problems such as difficulty with intubation and prolonged neuromuscular paralysis [1–3].

Diclofenac is a potent inhibitor of prostaglandin synthesis and belongs to the group of non-steroidal anti-inflammatory drugs (NSAID) [4]. I.m. diclofenac 75 mg is known to be efficient for the relief of pain in adults [4]. It has been shown also that diclofenac, administered i.m. as single doses of

50–100 mg daily, is an effective analgesic for dental or minor surgical pain, postpartum pain and headache [4]. Absorption is rapid after i.m. administration, with peak plasma concentrations occurring in about 10–30 min [4].

In this study we have evaluated the prophylactic effect of diclofenac on suxamethonium-induced myalgia and on analgesia (met-enkephalin-like activity (E-LA)) and pain mediators (prostaglandin E₂-like activity (PGE₂-LA), leukotriene C₄-like activity (LTC₄-LA) and histamine-like activity (H-LA) of plasma).

PATIENTS AND METHODS

We studied 34, ASA I adult patients undergoing elective ophthalmic surgery (blepharoplasty, dacryocystorhinotomy and plastic repair of the socket). Exclusion criteria included suspected neuromuscular, hepatic or neural disease, anticoagulant therapy, abnormal bleeding tendency, a history of peptic ulceration, known sensitivity to diclofenac and concurrent analgesic medication. Patients gave informed verbal consent to the study, which was approved by our Ethics Committee.

Patients were allocated randomly to two groups. Group 1 ($n = 17$) received saline 2 ml i.m. as premedication and group 2 ($n = 17$) received diclofenac (Voltaren, Ciba-Geigy) 75 mg (≈ 1.5 mg kg⁻¹) i.m. in the gluteal muscle 20 min before operation. The study was conducted in a double-blind fashion. A venous blood sample (baseline) was obtained before this premedication.

A standard anaesthetic technique was used in all patients. General anaesthesia was induced with thiopentone 5–7 mg kg⁻¹ and maintained with 67% nitrous oxide and halothane (end-tidal concentration 0.5%) in oxygen. A second blood sample (post-suxamethonium) was obtained 1 min after a bolus dose of suxamethonium 1.5 mg kg⁻¹ and the trachea was immediately intubated. A non-depolarizing neuromuscular blocking drug of appropriate duration of action was administered when controlled ventilation of the lungs was required.

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The intensity of visible fasciculations after suxamethonium was graded as: nil (absent); mild (fine fasciculations of the eyes, face, neck or fingers without movement of the limbs); moderate (obvious muscle twitching at more than two sites or movement of limb); severe (vigorous, sustained and widespread fasciculations). Intubation conditions were graded as: excellent (easy and no reaction); adequate (vocal cord movements present); fair (vocal cord movements and moderate reaction); poor (impossible without supplementation).

Blood samples for analysis of plasma PGE₂-LA, LTC₄-LA, H-LA and E-LA were obtained before premedication, 1 min after administration of suxamethonium, and 24 h after operation. Within 1 min of being taken they were centrifuged at 4 °C. Rat fundus muscles were used for assay of PGE₂-LA and guineapig ileal smooth muscles were used for the assays of LTC₄-LA, E-LA and H-LA.

Patients were visited 24 h after operation and questioned about the degree of myalgia, which was graded as: nil (absence of pain); mild (muscle stiffness or pain (on specific questioning) in the nape of the neck, shoulders, and lower chest on deep breathing); moderate (muscle stiffness and pain complained of by the patient spontaneously, requiring analgesia); severe (incapacitating generalized muscle stiffness or pain).

Surgeons were asked about any intra- and post-operative bleeding or other side effects. Post-operative nausea, vomiting, local reaction or pain at injection site, analgesic requirements and mobility were also recorded.

Data were analysed by analysis of variance. Intubating condition, fasciculation and myalgia scores were analysed by Mann-Whitney *U* test. *P* < 0.05 was considered significant.

RESULTS

There was no significant difference between patient characteristics in the two groups (*P* > 0.05) (table I).

Premedication with diclofenac was associated with a significant reduction in the intensity of myalgia, but not the intensity of visible muscle fasciculations (*P* < 0.05). There was no correlation between the degree of visible fasciculations and the occurrence of muscle pain (*P* > 0.05). Among the patients given diclofenac, only one (5.9%) felt severe pain 24 h after operation, one (5.9%) had moderate pain, six

(35.3%) had mild pain and nine (52.9%) had no pain. Three (17.7%) patients in the control group had severe pain, four (23.5%) had moderate pain, six (35.3%) had mild pain and four (23.5%) were free of pain (fig. 1).

Intubating conditions were poor in two (11.8%) patients, adequate in one (5.9%) and excellent in 14 (82.3%) in the control group; poor in one (5.9%), fair in one (5.9%) and excellent in 15 (88.2%) patients in the diclofenac group. These differences were not significant (*P* > 0.05).

Plasma PGE₂-LA was increased significantly in the control group, from 12.91 (SE 1.6) ng ml⁻¹ (baseline) to 15.21 (2.3) ng ml⁻¹ (*P* < 0.05) after suxamethonium and to 16.13 (2.7) ng ml⁻¹ (*P* < 0.05) at 24 h after operation. In the diclofenac group there was an insignificant increase after suxamethonium (from 12.75 (2.0) ng ml⁻¹ to 14.60 (2.4) ng ml⁻¹) (*P* > 0.05) which remained almost unchanged (*P* > 0.05) 24 h after operation.

Plasma LTC₄-LA was also increased significantly in the control group, from the baseline value of 8.17 (1.4) ng ml⁻¹ to 10.47 (2.0) ng ml⁻¹ (*P* < 0.05) after suxamethonium, but decreased to 8.76 (1.5) ng ml⁻¹ 24 h after operation. Plasma LTC₄-LA in the diclofenac group was initially 7.44 (1.9) ng ml⁻¹, increased to 8.33 (1.5) ng ml⁻¹ after suxamethonium and decreased to 7.5 (1.5) ng ml⁻¹ (not significant).

Plasma H-LA was increased in both groups after administration of suxamethonium. While the increase (from 26.8 (2.8) ng ml⁻¹ to 31.9 (2.8) ng ml⁻¹) in the control group was significant (*P* < 0.05), that in the diclofenac group (from 31.0 (5.1) ng ml⁻¹ to

TABLE I. Patient characteristics (mean (range or SD))

	Group 1 (Control)	Group 2 (Diclofenac)
<i>n</i>	17	17
Age (yr)	33.47 (20-59)	31.13 (23-50)
Weight (kg)	58.33 (15.8)	59.13 (14.0)
Sex (M/F)	7/10	9/8
Operation		
Blepharoplasty	8	7
Dacryocystorhinotomy	8	8
Plastic repair of the socket	1	2
Duration of operation (min)	40.6 (14.1)	33.7 (13.5)
Duration of anaesthesia (min)	63.0 (18.9)	55.7 (17.6)

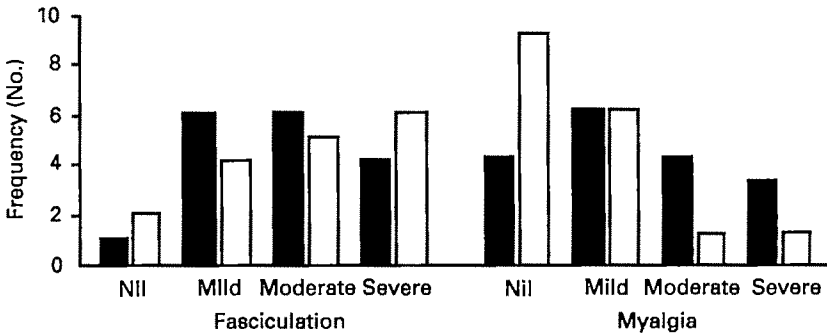


FIG. 1. Frequency of visible muscle fasciculations and postoperative myalgia after administration of suxamethonium in group 1 (control) (■) and group 2 (diclofenac) (□) (mean, SE).

34.6 (4.2) ng ml⁻¹) was not. H-LA values after operation (30 (2.6) ng ml⁻¹ in the control group; 30.7 (3.1) ng ml⁻¹ in the diclofenac group) were not significantly different from baseline in both groups.

Baseline plasma E-LA in the control and diclofenac groups were 2.24 (0.4) ng ml⁻¹ and 2.0 (0.3) ng ml⁻¹, respectively, increasing to 2.79 (0.5) ng ml⁻¹ and 2.32 (0.3) ng ml⁻¹, respectively, after suxamethonium ($P < 0.005$ both groups). At 24 h after operation, values had returned to baseline in the diclofenac group (2.23 (0.4) ng ml⁻¹), but remained increased (2.68 (0.5) ng ml⁻¹ ($P < 0.05$)) in the control group.

Differences in values of each mediator (plasma PGE₂-LA, LTC₄-LA, H-LA and E-LA) between the control and diclofenac groups were not significant ($P > 0.05$).

No patient received any analgesic or opioid drug during the anaesthetic and postoperative period and administration of diclofenac was not associated with bleeding or side effects. None of the patients complained of pain at the injection site. On the first night after operation, all patients were confined to bed and the next morning they were permitted to mobilize. All were fully active within 30 h and discharged within 48 h.

DISCUSSION

The most common side effect of suxamethonium is probably muscle pain which resembles the aches experienced after unaccustomed exercise.

Although there is often biochemical evidence of muscle damage, with increased plasma concentrations of creatinine kinase and myoglobin after administration of suxamethonium [5, 6], no obvious relationship between pain and biochemical changes has been found [5, 7]. There is also no direct correlation between visible muscle fasciculations and incidence of muscle pains [1, 8]. Collier has reported previously a transient decrease in the serum concentration of calcium 1 min after administration of suxamethonium to patients who experienced muscle pains. He postulated that influx of calcium into the muscle caused an increase in muscle damage and pain [9]. Jackson, Jone and Edwards showed, *in vitro*, that excessive repetitive contractile activity was associated with increased calcium uptake, activation of phospholipase A₂, generation of arachidonic acid and synthesis of prostaglandins [10] which may induce delayed-onset inflammation [11, 12]. McLoughlin, Nesbitt and Howe reported a significant reduction in suxamethonium-induced myalgia with soluble aspirin 600 mg given 1 h before operation, and suggested that prostaglandins were responsible for the pain [13]. However, studies from Jackson's laboratory have suggested that lipo-oxygenase products are mediators of calcium-induced intracellular enzyme efflux from skeletal muscle [10, 14], whereas myalgia may be mediated by cyclo-oxygenase products.

In this study, we found that preoperative administration of diclofenac 75 mg reduced suxamethonium-induced muscle pain significantly and did not interfere with intubating conditions. There

was no correlation between myalgia and the extent of visible fasciculations. Although patients in the diclofenac group appeared to have more severe fasciculations, this was not statistically significant.

Blood sampling times were chosen as 1 min after administration of suxamethonium (at which time a significant decrease in plasma calcium concentration has been demonstrated [9]) and 24 h after operation (when muscle pains are known to be manifest and a marked increase in plasma creatinine kinase concentration has been reported [5]).

Prostaglandins, leukotrienes and histamine are known to potentiate nociceptive agents and also the formation of oedema [11]. Small doses of PGE₂, in particular, appear to sensitize the pain receptors to stimulation by touch, histamine or bradykinin [15]. PGE₂ also potentiates the oedema-producing capacity of histamine and bradykinin [11, 16]. We observed an increase of 17% in plasma PGE₂-LA and 28% in plasma LTC₄-LA after suxamethonium in the control group in whom there was a high incidence of myalgia. The comparable increase in these substances was not significant in the treatment group, showing a protective effect of diclofenac against a suxamethonium-induced inflammatory response, although the correlation between myalgia and changes in PGE₂-LA and LTC₄-LA does not imply a direct cause-effect relationship. We observed similar changes in both LTC₄-LA and PGE₂-LA; as reported previously, NSAID may block both cyclo-oxygenase and lipo-oxygenase pathways [4, 12]. The protective effect of diclofenac was only partial, suggesting that other substances, such as histamine which was increased in both groups, may affect vasodilatation and oedema formation. This increase in plasma H-LA after administration of suxamethonium was significant in the control group, suggesting an effect of the relatively large concentrations of prostaglandins which have been suggested previously to release histamine from mast cells [17].

Noxious stimulation is known to be an important activator of the normally quiescent endogenous pain suppression system [18, 19], causing direct stimulation of peripheral β -endorphin [20, 21] and met-enkephalin [22]. It has been suggested that excessive secretion of endogenous opioids in response to pain and stress might be associated with "spill-over" of opioid peptides into the systemic circulation, with the possibility that their concentration in plasma may be taken as an indicator [23]. In agreement with these studies, we observed a significant increase in plasma E-LA after induction of anaesthesia in both groups. The persisting increased enkephalin-like activity in the control group after operation may be explained by the relatively great concentrations of the potent hyperalgesic mediators, PGE₂ and LTC₄.

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ACCELERATED CONTRACTURES AFTER ADMINISTRATION OF RYANODINE TO SKELETAL MUSCLE OF MALIGNANT HYPERTHERMIA SUSCEPTIBLE PATIENTS

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SUMMARY

A genetic disorder of the calcium releasing ryanodine receptor has recently been postulated in malignant hyperthermia (MH) and ryanodine-induced contractures differ between subjects who are malignant hyperthermia susceptible (MHS) and non-susceptible (MHN). We tested 39 patients from 26 families for MH, using the procedure of the European Malignant Hyperthermia Group. A ryanodine contracture test was performed by both cumulative ($0.4\text{--}10.0\ \mu\text{mol litre}^{-1}$ every 3 min) and bolus ($10.0\ \mu\text{mol litre}^{-1}$) application. Contracture with cumulative ryanodine application started significantly earlier in MHS ($9.6\ (\text{SEM } 0.5)\ \text{min}$) than in MHN patients ($24.6\ (1.3)\ \text{min}$). A significant difference in start of contracture between MHS ($4.8\ (0.6)\ \text{min}$) and MHN ($14.5\ (0.6)\ \text{min}$) patients occurred also after bolus application of ryanodine. The ryanodine contracture test seems to be a potentially specific *in vitro* diagnostic test for MH. (Br. J. Anaesth. 1993; 71: 242–246)

KEY WORDS

Hyperthermia: malignant, ryanodine contracture test.

A sudden increase in myoplasmic calcium concentration is thought to be the initial event in the hypermetabolic reaction of a malignant hyperthermia (MH) crisis. The genetic disorder of MH is therefore thought to be in calcium regulation [1–4]. The ryanodine receptor has been shown to be identical with the calcium release channel of the sarcoplasmic reticulum (SR) [5–8]. In swine, the ryanodine receptor is different in MH-susceptible (MHS) and non-susceptible (MHN) individuals [9–14]. In humans, the genetic localization of the ryanodine receptor is in the MHS region of gene 19-q12-13.2. [15, 16]. It was reported recently that ryanodine-induced contractures are able to differentiate between MHS and MHN individuals, and therefore might provide a potentially specific *in vitro* diagnostic test for MH [17, 18].

PATIENTS AND METHODS

We studied 39 patients from 26 families (21 male and 18 female; 33 adults and six children, ages 6–61 yr) with clinically suspected MH. After physical ex-

amination, a personal and family history, and written consent for the different tests were obtained from all patients. Plasma creatinine kinase concentration was measured after a 24-h resting period. Adult muscle biopsies were obtained under regional anaesthesia (3-in-1 block) with 1% prilocaine 40 ml as outpatients. Child muscle biopsies were obtained under general anaesthesia with alfentanil, midazolam, nitrous oxide and oxygen. Two or three muscle bundles were excised carefully, avoiding trauma. The excised muscle samples were placed in a container filled with Krebs–Ringer solution equilibrated with Carbogen (95% oxygen–5% carbon dioxide) and transported to the laboratory immediately. The specimens were divided into four to eight strips approximately $15\text{--}25\ \text{mm} \times 2\text{--}3\ \text{mm}$ and weighing approximately 80–200 mg. All areolar tissue and fat were removed carefully.

The muscle strip was secured with silk sutures to a fixed point and a force displacement transducer (TF 3V 100 inductiv, W. Fleck, Mainz, Germany). The whole system was placed in a 80-ml organ bath. Isometric tension was amplified (Gould, Cleveland, Ohio, U.S.A.) and recorded with a polygraph (TA 200, Gould). Supramaximal electrical stimulation via platinum electrodes was undertaken with a square-wave stimulator (Grass Stimulator SD 9, Quincy, Mass., U.S.A.), using a duration of 1 ms and frequency of 0.2 Hz. The initial baseline tension was set at 2 g. Two muscle strips were investigated simultaneously.

After at least a 10-min period of steady baseline recording, halothane $0.11\text{--}0.22\text{--}0.44\ \text{mmol litre}^{-1}$ or caffeine $0.5\text{--}1.0\text{--}1.5\text{--}2.0\text{--}3.0\text{--}4.0\text{--}32.0\ \text{mmol litre}^{-1}$ was added according to the procedure of the European Malignant Hyperthermia Group (EMHG), incrementally every 3 min. Ryanodine was also added incrementally ($0.4\text{--}0.8\text{--}1.6\text{--}10.0\ \mu\text{mol litre}^{-1}$) every 3 min or as a bolus ($10.0\ \mu\text{mol litre}^{-1}$). The organ bath with ryanodine was observed for at least 70 min after maximal concentration.

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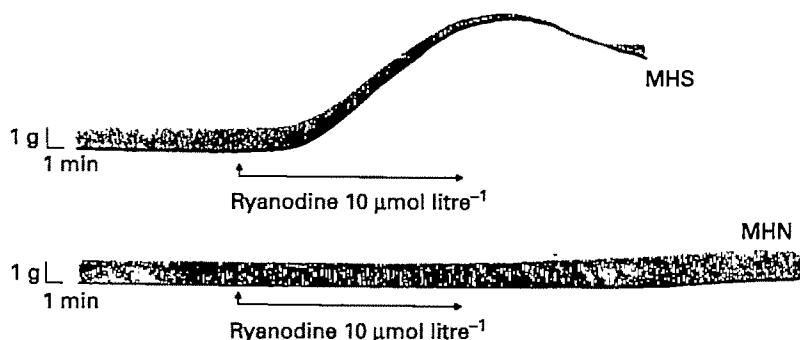


FIG. 1. Effects of ryanodine bolus application ($10.0 \mu\text{mol litre}^{-1}$) on contracture development in skeletal muscle preparations of MH-susceptible (MHS, upper curve) and MH non-susceptible (MHN, lower curve) patients. (Original recordings of a representative specimen.)

The *in vitro* caffeine-halothane contracture (CHC) test gave the caffeine and halothane threshold for each patient. Patients were classified according to the criteria of the EMHG as follows:

MHS = A caffeine threshold at a caffeine concentration of $2.0 \text{ mmol litre}^{-1}$ or less, and a halothane threshold concentration at $0.44 \text{ mmol litre}^{-1}$ or less.
MHN = A caffeine threshold at a caffeine concentration of $3 \text{ mmol litre}^{-1}$ or more and a halothane threshold concentration greater than $0.44 \text{ mmol litre}^{-1}$.

MHE = All other results deemed equivocal.

For the ryanodine groups, three contracture points were established as a threshold time and not as a threshold concentration: the start of contracture, a contracture of 0.2 g and a contracture of 1.0 g .

Composition of the Krebs-Ringer solution was (mmol litre^{-1}): NaCl 118.1; KCl 3.4; CaCl_2 2.5; MgSO_4 0.8; KH_2PO_4 1.2; NaHCO_3 25.0; glucose 11.1. Temperature was constant at 37°C , pH was 7.4, and carbogen was bubbled into each organ bath continuously.

The following chemicals were used: caffeine (Sigma Chemie, Deisenhofen, Germany), halothane (Hoechst AG, Frankfurt, Germany), ryanodine (Merck, Sharpe and Dohme, Rahway, N.J., U.S.A.). All other substances were "pro analysi" or best purity available. Solutions were mixed every day in carbonated Krebs-Ringer solution at 37°C and added directly to the organ bath. Halothane was added to the carbogen from a calibrated vaporizer (Dräger, Lübeck, Germany). Halothane concentration was measured with an anaesthetic gas monitor (Normac, Datex, Helsinki, Finland). Bath concentration of halothane was determined by gas chromatography.

All data are presented as mean (SEM). Statistical analysis was by Student's *t* test for unpaired samples. Results were considered significant at $P < 0.05$.

RESULTS

Twenty-three subjects were classified as MHS and 10 as MHN by EMHG criteria. Figure 1 shows a typical MHS and MHN muscle contracture curve. After ryanodine bolus application ($10.0 \mu\text{mol litre}^{-1}$) the start of contracture was earlier in the MHS than in the MHN muscle specimen. The results from

individual contracture curves are shown in tables I and II.

In the MHS group, contracture with cumulative ryanodine started after 9.6 min and reached 0.2 g after 18.2 min and 1.0 g after 27.9 min. These contractures were reached significantly later in the MHN group: 24.6 min, 36.8 min and 45.0 min, respectively. Start of contracture occurred between 5.5 and 14.0 min in the MHS group and between 20.5 and 31.5 min in the MHN group. However, when contracture reached 0.2 g or 1.0 g , there was a small overlap between groups (table I).

During cumulative application of ryanodine, muscle contracture started at a time when the maximum concentration of ryanodine ($10.0 \mu\text{mol litre}^{-1}$) had already been added to the organ bath. Thus it was presumed that an incubation time of 3 min for each concentration was not enough to reach maximum effect, and in additional experiments, a bolus application of ryanodine $10.0 \mu\text{mol litre}^{-1}$ was made. Both MHS and MHN groups attained contractures significantly earlier after bolus than after cumulative application: at 1.0–11.0 min in the MHS group and at 12.0–17.0 min in the MHN group (table I). Again, there was a small overlap between groups when contractures reached 0.2 g or 1.0 g (table I).

Table II shows individual data for six MHEc patients. In four patients, both a cumulative and a bolus ryanodine contracture test were performed; in two only a bolus application of ryanodine was performed. Using the range of values given in table I, patient No. 2 could be classified as MHS, patient No. 4 as MHN and the remainder as equivocal with the cumulative ryanodine test. With the bolus test, patients Nos 2, 3, 5 and 6 could be classified as MHS and patients Nos 1 and 4 as MHN.

DISCUSSION

The primary defect in MH is thought to be in calcium regulation, at least of skeletal muscle [1, 2]. The defect is located in the calcium releasing channel of the sarcoplasmic reticulum [3, 4]. Biochemical investigations have shown abnormal changes in the ryanodine receptor of MHS pigs compared with MHN [9–11, 13, 14]. Fujii and colleagues [9] showed a single point mutation in the porcine ryanodine

TABLE I. Contracture course and thresholds after cumulative ($0.4\text{--}10.0\ \mu\text{mol litre}^{-1}$ every 3 min) and bolus application ($10.0\ \mu\text{mol litre}^{-1}$) of ryanodine in skeletal muscle preparation of malignant hyperthermia susceptible (MHS) and non-susceptible (MHN) patients. Contractures: Begin = beginning of contracture; 0.2 g = contracture reaches 0.2 g; 1.0 g = contracture reaches 1.0 g; n.d. = not determined. Hal. = halothane; Caff. = caffeine. * $P < 0.05$ MHS vs MHN

Patient No.	Cumulative			Bolus			Threshold (mmol litre^{-1})	
	Begin (min)	0.2 g (min)	1.0 g (min)	Begin (min)	0.2 g (min)	1.0 g (min)	Hal.	Caff.
MHS								
1	n.d.	n.d.	n.d.	6.0	10.0	15.0	0.11	1.0
2	10.0	19.0	33.0	2.0	9.0	21.0	0.22	1.5
3	9.5	11.0	13.5	1.0	2.5	4.0	0.22	1.0
4	10.5	20.5	24.5	11.0	15.0	21.0	0.22	1.5
5	12.0	20.0	31.0	5.0	12.5	23.0	0.44	2.0
6	8.0	17.0	26.0	6.0	11.0	18.0	0.22	1.5
7	14.0	22.0	37.0	7.0	14.5	24.0	0.44	1.5
8	8.0	10.0	12.5	1.5	4.0	5.5	0.11	0.5
9	8.0	15.0	38.0	2.5	21.0	34.0	0.44	0.5
10	n.d.	n.d.	n.d.	10.0	16.0	23.0	0.44	2.0
11	10.0	18.5	27.0	1.5	12.0	17.5	0.44	1.0
12	8.0	17.5	25.0	3.0	7.0	12.5	0.44	1.0
13	8.0	17.5	27.5	6.0	10.5	24.0	0.22	1.5
14	11.0	18.0	27.5	2.0	11.5	26.0	0.22	1.5
15	11.0	16.5	24.0	6.0	14.0	23.0	0.44	1.0
16	10.0	23.0	41.0	10.0	21.0	25.0	0.44	1.0
17	10.0	17.0	25.0	2.0	12.0	27.0	0.44	2.0
18	6.0	17.5	25.5	6.0	10.0	16.5	0.22	1.5
19	5.5	25.5	37.0	1.0	10.5	30.0	0.22	1.5
20	12.0	22.0	28.0	5.0	12.0	30.0	0.44	1.0
21	n.d.	n.d.	n.d.	7.0	12.0	18.0	0.22	2.0
22	n.d.	n.d.	n.d.	6.0	16.0	30.5	0.44	1.5
23	n.d.	n.d.	n.d.	3.5	9.5	17.5	0.44	2.0
Mean	9.6*	18.2*	27.9*	4.8*	11.9*	21.9*		
SEM	0.5	0.9	1.8	0.6	0.9	0.9		
MHN								
1	22.5	27.5	42.5	n.d.	n.d.	n.d.	—	3.0
2	23.0	57.0	—	n.d.	n.d.	n.d.	—	3.0
3	31.5	43.0	60.0	16.5	21.0	27.0	—	3.0
4	22.5	29.5	37.5	13.5	19.5	37.0	—	3.0
5	25.0	34.0	49.0	13.0	19.0	31.0	—	3.0
6	20.5	23.5	—	16.0	21.0	30.5	—	3.0
7	28.0	52.0	—	12.0	16.0	34.0	—	4.0
8	24.0	28.0	36.0	17.0	25.0	45.0	—	3.0
9	n.d.	n.d.	n.d.	13.5	18.0	24.5	—	3.0
10	n.d.	n.d.	n.d.	14.5	20.5	28.5	—	3.0
Mean	24.6	36.8	45.0	14.5	20.0	32.2		
SEM	1.3	4.4	4.4	0.6	0.9	2.3		

TABLE II. Contracture course and thresholds after cumulative ($0.4\text{--}10.0\ \mu\text{mol litre}^{-1}$ every 3 min) and bolus application ($10.0\ \mu\text{mol litre}^{-1}$) of ryanodine in skeletal muscle preparation of malignant hyperthermia equivocal (MHE) patients. Contractures: Begin = beginning of contracture; 0.2 g = contracture reaches 0.2 g; 1.0 g = contracture reaches 1.0 g. Hal. = halothane; Caff. = caffeine.

Patient No.	Cumulative			Bolus			Threshold (mmol litre^{-1})	
	Begin (min)	0.2 g (min)	1.0 g (min)	Begin (min)	0.2 g (min)	1.0 g (min)	Hal.	Caff.
MHEc								
1	18.0	23.0	33.0	15.0	20.0	27.0	—	2.0
2	8.0	26.0	37.0	3.0	10.0	25.0	—	2.0
3	17.5	21.5	24.5	6.0	17.5	28.5	—	1.0
4	30.0	36.5	63	22.0	34.0	—	—	2.0
5	n.d.	n.d.	n.d.	6.0	14.5	21.5	—	2.0
6	n.d.	n.d.	n.d.	6.5	11.5	17.0	—	1.5

receptor associated with MH, and genetic linkage studies indicate that a single point mutation of the ryanodine receptor may occur in humans [15, 16, 19]. However, other studies suggest that the ryanodine receptor gene is not always responsible for MH susceptibility [20–26]. The calcium release channel has been shown to be identical with the ryanodine receptor [5–8], but the exact mechanism of

ryanodine-induced calcium release from SR is still unknown [4, 11, 13, 26, 27]. The differences could be explained partly by concentration-dependent action at the receptor site: small concentrations would open and large concentrations ($> 10\ \mu\text{mol litre}^{-1}$) would close calcium release channels. Further, calcium and ATP activate the channels, whereas magnesium and calmodulin inactivate them

[6]. A second messenger such as inositol trisphosphate (IP₃) may mediate ryanodine receptor function [24, 28–30].

The present study has shown that both cumulative and bolus application of ryanodine results in a significantly earlier development of contracture in muscle biopsies in MHS compared with MHN patients. Hopkins, Ellis and Halsall [17] noted contractures during cumulative application of ryanodine in the MHS group, and none in the MHN group. We demonstrated that contracture developed only with the maximum concentration of ryanodine. A possible explanation for this contradictory finding could be that ryanodine from two different companies was used, having different purity and proportion of the isomers, ryanodine and 9,21-dehydroryanodine. Both studies enabled all patients to be classified as MHS and MHN in accordance with the CHC test.

In this study, a bolus application of ryanodine 10.0 µmol litre⁻¹ was used. Contracture in MHS patients again developed significantly earlier than in MHN patients and all patients could be classified as MHS or MHN. Because ryanodine is highly toxic (LD₅₀ of less than 50 mg kg⁻¹ in mammals), it may destroy the cells during cumulative application. Thus bolus application seems to be superior and was able to categorize MHE patients as MHS or MHN. In a previous study [17], it was possible to classify two MHEh patients as MHS using the ryanodine contracture test. Although one has to be careful in interpreting the data of the previous and present study because of the small numbers of patients, the ryanodine contracture test with bolus application seems to be a potentially specific *in vitro* diagnostic test for MH classification.

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ISOFLURANE REDUCES MICROTUBULE-ASSOCIATED PROTEIN 2 DEGRADATION COMPARED WITH HALOTHANE DURING FOREBRAIN ISCHAEMIA IN THE RAT

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SUMMARY

One of the most prominent features of the early phase of cerebral ischaemia is the immunohistochemical collapse of cytoskeletal proteins. Among these proteins, microtubule-associated protein 2 (MtP2) has been shown to be vulnerable to ischaemic injuries. In order to identify a suitable volatile anaesthetic on the basis of cytoskeletal protein breakdown during cerebral ischaemia, we have compared the effects of isoflurane and halothane on MtP2 degradation in rats. Under equipotent isoflurane or halothane anaesthesia, forebrain ischaemia was induced by occlusion of the bilateral common carotid artery, combined with a decrease in mean arterial pressure to 50 mm Hg. After 20 min of ischaemia, the frontoparietal cortex, brainstem, hippocampus and cerebellum were removed separately and homogenized. MtP2 from each region was measured using an enzyme-linked immunosorbent assay. MtP2 degradation in the frontoparietal cortex and hippocampus was significantly ($P < 0.05$ and $P < 0.01$) less with isoflurane anaesthesia (75.6 (SD 10.7)% and 72.3 (12.8)%, respectively) than with halothane (65.0 (13.1)% and 54.7 (13.9)%, respectively). (Br. J. Anaesth. 1993; 71: 247–252)

KEY WORDS

Anaesthetics volatile: halothane, isoflurane. Brain: ischaemia.

During ischaemia, the most vulnerable regions of the brain are the subiculum-CA1 region of the hippocampus, some layers of the cerebral cortex and the Purkinje cell layer of the cerebellum [1]. In these regions, a few minutes of cerebral ischaemia selectively induces neuronal cell loss several days after the ischaemic insult [2, 3]. In order to evaluate the outcome of cerebral ischaemia histopathologically, it is necessary to wait for several days after the ischaemic insult because the evolution of irreversible ischaemic injuries is preceded usually by a long interval during which morphological changes are subtle or absent [4].

In contrast, one phenomenon which occurs during the early phase of cerebral ischaemia is immunological collapse of cytoskeletal proteins [5, 6]. Among these, microtubule-associated protein 2 (MtP2),

which is localized typically in dendrites and neuronal cell bodies [7, 8], has been shown immunohistochemically to be extremely vulnerable to ischaemic injury [5, 9]. Therefore, detection of MtP2 degradation immediately after cerebral ischaemia is an early and sensitive marker for cerebral ischaemic damage, as the post-synaptic sites, the dendrites and neuronal cell bodies, are the most susceptible to the ischaemic insult [10].

The purpose of this study was to compare the effects of isoflurane and halothane on MtP2 degradation during 20 min of incomplete forebrain ischaemia in the rat.

METHODS

This study was performed in accordance with the ethical principles provided by the Experimental Animal Laboratory of Gunma University School of Medicine.

Thirty-five male Wistar rats (SLC, Hamamatsu, Japan), weights 270–350 g, were studied. The animals were allowed to eat and drink freely until the start of the investigation. Under equi-MAC (minimum alveolar concentration) isoflurane or halothane in oxygen anaesthesia, forebrain ischaemia was induced by occlusion of the bilateral common carotid artery (CCA), combined with a reduction in mean arterial pressure (MAP) to 50 mm Hg by bleeding [11]. After induction of anaesthesia, both CCA were exposed and ligatures placed around them, carefully avoiding the vagus nerves and cervical sympathetic plexi. Tracheotomy was performed and, after administration of pancuronium 0.1 mg i.p., the lungs were ventilated using a small animal ventilator (Model SN-480-7; Shinano Manufacturing Company) which delivered a gas mixture of 66% nitrogen in oxygen and 1.0 MAC isoflurane or halothane (approximately 1.4% and 1.1%, respectively, in rats [12]) at a rate which maintained arterial carbon dioxide partial pressure (P_{aCO_2}) at about 4.7–5.3 kPa. The femoral artery was cannulated with a 24-gauge Teflon catheter and connected to a computer-linked continuous arterial pressure monitoring system (AP-

TABLE I. *Physiological variables (mean (SD)) in the three groups (see text for details). **P < 0.01 between isoflurane and halothane*

	Occlusion group		Hypotension group		Combined group	
	Isoflurane	Halothane	Isoflurane	Halothane	Isoflurane	Halothane
Number of rats	5	5	5	5	5	5
Body weight (g)	299 (22.2)	324 (48.9)	322 (29.7)	303 (28.4)	313 (29.1)	291 (31.9)
Just before start of ischaemic insult						
MAP (mm Hg)	115 (3.6)	116 (7.9)	123 (16.9)	123 (12.3)	116 (3.2)	127 (15.0)
pH	7.39 (0.04)	7.43 (0.08)	7.38 (0.02)	7.40 (0.05)	7.40 (0.05)	7.41 (0.03)
P_{aCO_2} (kPa)	5.2 (0.5)	4.9 (0.5)	5.5 (0.6)	5.2 (0.9)	5.2 (0.5)	4.9 (0.4)
P_{aO_2} (kPa)	19.5 (2.8)	18.7 (5.8)	22.5 (1.4)	24.5 (4.1)	18.6 (3.4)	19.1 (4.2)
Base excess (mmol litre ⁻¹)	-1.3 (2.7)	-1.5 (3.0)	-0.7 (0.7)	-0.8 (1.7)	-0.8 (3.4)	0 (1.5)
PCV (%)	39.2 (3.0)	40.4 (2.7)	39.2 (3.0)	39.8 (1.5)	39.0 (3.1)	41.2 (4.0)
Blood glucose (mg dl ⁻¹)	222 (72.7)	197 (72.1)	231 (57.8)	241 (25.1)	244 (44.4)	192 (45.7)
Rectal temperature (°C)	37.1 (0.1)	37.0 (0.2)	36.9 (0.2)	37.0 (0.1)	36.9 (0.2)	37.0 (0.2)
15 min after start of ischaemic insult						
MAP (mm Hg)	114 (9.4)	122 (8.6)	47 (2.0)	49 (2.4)	48 (2.8)	49 (3.1)
pH	7.39 (0.04)	7.41 (0.06)	7.38 (0.02)	7.38 (0.05)	7.37 (0.07)	7.38 (0.07)
P_{aCO_2} (kPa)	5.2 (0.5)	4.9 (0.8)	4.7 (0.4)	4.7 (0.3)	4.6 (0.2)	4.6 (0.2)
P_{aO_2} (kPa)	18.3 (2.9)	16.9 (5.6)	21.8 (1.7)	25.8 (5.4)	19.3 (3.7)	20.3 (3.7)
Base excess (mmol litre ⁻¹)	-0.9 (2.1)	-0.9 (1.9)	-4.9 (2.6)	-4.9 (2.3)	-4.6 (3.5)	-5.3 (3.7)
PCV (%)	38.2 (3.1)	40.8 (2.6)	31.2 (3.6)	33.6 (2.4)	32.8 (4.1)	33.2 (5.4)
Blood glucose (mg dl ⁻¹)	276 (65.7)	230 (69.1)	317 (76.0)	320 (48.1)	356 (26.7)**	274 (28.5)
Rectal temperature (°C)	36.9 (0.1)	37.1 (0.2)	37.0 (0.4)	37.0 (0.1)	37.0 (0.1)	37.1 (0.2)
Termination of ischaemic insult						
Shed blood (ml kg ⁻¹)	2.8 (0.4)	2.7 (1.1)	15.9 (3.2)	20.5 (5.1)	18.6 (5.9)	16.3 (4.7)

601G; Nihon Koden). The femoral vein was cannulated with a polyethylene catheter (PE-50) to administer heparin 50 iu and to withdraw or re-infuse blood to maintain MAP at 50 mm Hg. Lactated Ringer's solution was infused continuously with a syringe pump (Model STC-521; Terumo) at a rate of 5 ml kg⁻¹ h⁻¹. The animals were allowed to attain a steady state during a period of about 30 min. Rectal temperature was maintained at 37 °C using an animal temperature controller (CMA/150; CMA). The output of the vaporizer was confirmed using an inhalation anaesthetic gas monitor (Capnomac Ultima; Datex).

Forebrain ischaemia was induced by a combination of bilateral tightening of the ligature and simultaneous reduction in MAP to 50 mm Hg by withdrawal of blood from a femoral vein. To verify if forebrain ischaemia had occurred, the extracranial electroencephalogram (EEG) of the frontoparietal region was recorded using an EEG monitor (Neuropack-II; Nihon Koden).

The animals were allocated to a control group (five rats) and three ischaemic groups (six subgroups of five rats each). In the control group, no ischaemic insult was given. In the occlusion group, bilateral CCA occlusion was performed without decreasing MAP, during either isoflurane or halothane anaesthesia. In the hypotension group, MAP was decreased to 50 mm Hg without CCA occlusion (haemorrhagic hypotension), during either isoflurane or halothane anaesthesia. In the combined group, bilateral CCA occlusion was combined with decreased MAP to 50 mm Hg, during either isoflurane or halothane anaesthesia. In each group except the control, the duration of ischaemic time was 20 min. The start of the ischaemic period was defined as the time that the ligature around the bilateral CCA was

tightened in the occlusion group and the time that MAP first reached 50 mm Hg in the hypotension group and combined group. In order to measure physiological variables, a small amount of arterial blood was obtained both just before and 15 min after the initiation of the ischaemic insult.

After 20 min of ischaemia, decapitation was performed and the frontoparietal cortex, brainstem, hippocampus and cerebellum removed immediately and homogenized separately in buffer containing sucrose 0.32 mol litre⁻¹, Tris (tris (hydroxymethyl) aminomethane) HCl 10 mmol litre⁻¹ (pH 7.0), leupeptin 100 µmol litre⁻¹ and EGTA (ethyleneglycol bis (2-aminoethyl-ether) tetra-acetic acid) 1 mmol litre⁻¹, using a motor-driven glass homogenizer with a Teflon pestle [6]. Homogenates were stored at -80 °C until required for analysis. Protein concentrations were measured as described previously [13]. The amount of MtP2 in each region for each group was measured as the ratio to that of the control rat brain using an enzyme-linked immunosorbent assay (ELISA) [14-16]: solutions were prepared from each of the stored homogenates by dilution with phosphate-buffered saline (PBS), addition of one drop of surfactant (Triton X-100) and storage at 4 °C overnight. A 100-µl aliquot of each sample was transferred to a 96-well microplate (high-binding type, Costar), one drop of trichloroacetate was added and the microplate stored at 4 °C overnight. Subsequently, after centrifugation of the microplate at 500 g for 20 min, the sample solutions were washed with ethanol and PBS and 100 µl of the primary antibody (mouse anti-MtP2 monoclonal antibody: immunized against rat brain microtubule-associated protein, Chemicon; diluted 1000 times with PBS) was added to each well and the microplate incubated for about 1 h at 37 °C in a humid chamber. After

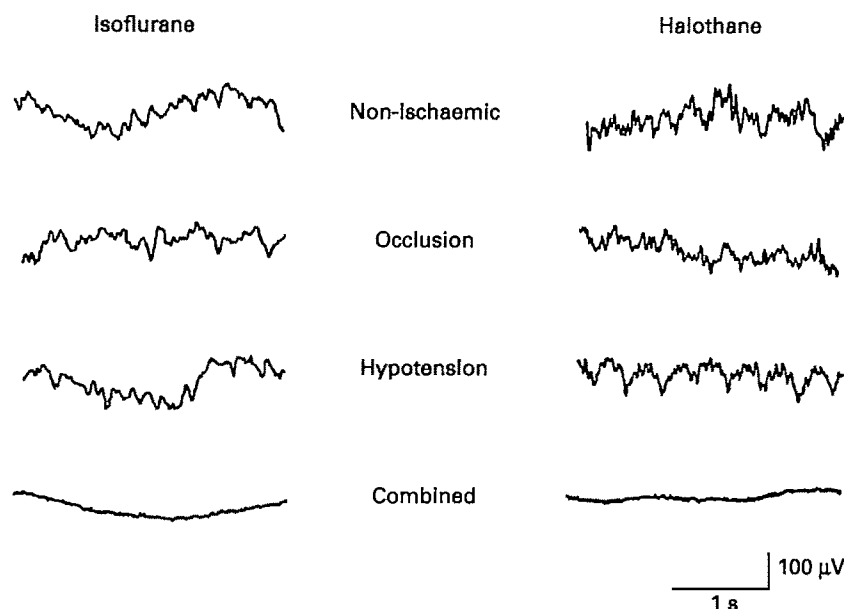


FIG. 1. Changes in the EEG recorded extracranially in the frontoparietal region. Compared with non-ischaemic states (just before initiation of the ischaemic insult), electrical activity in the occlusion and hypotension groups is well preserved, whereas the combined groups show complete electrical silence during both isoflurane and halothane anaesthesia.

TABLE II. MtP2 degradation during 20 min of forebrain ischaemia in the rat (mean (SD)). Significant differences: * $P < 0.05$ and ** $P < 0.01$ between isoflurane and halothane; † $P < 0.05$ compared with occlusion group; *** $P < 0.001$ compared with occlusion and hypotension groups. $n = 25$ for all results

	MtP2 degradation (%)	
	Isoflurane	Halothane
Frontoparietal cortex		
Occlusion	101.1 (10.6)	101.6 (9.6)
Hypotension	96.8 (11.6)	96.2 (9.5)
Combined	75.6 (10.7)***	65.0 (13.1)***
Brainstem		
Occlusion	102.1 (7.7)	98.2 (10.1)
Hypotension	95.5 (11.1)	95.2 (10.5)
Combined	93.6 (9.9)†	96.3 (8.8)
Hippocampus		
Occlusion	99.8 (10.2)	100.1 (8.1)
Hypotension	99.3 (10.6)	94.7 (10.6)
Combined	72.3 (12.8)***	54.7 (13.9)***
Cerebellum		
Occlusion	98.6 (12.3)	96.9 (12.5)
Hypotension	97.2 (11.2)	94.8 (15.2)
Combined	96.3 (12.6)	96.7 (12.5)

washing with PBS, the secondary antibody (horse-radish peroxidase-conjugated anti-mouse IgG; diluted 2000 times with PBS) was reacted with the primary antibody for about 1 h at 37 °C. After washing, 250 µl of the enzyme substrate (o-phenylenediamine dihydrochloride 1 mg ml⁻¹ + 0.5 % hydrogen peroxide) was added and reacted with the secondary antibody for about 15 min at 37 °C. The reaction was stopped by addition of 50 µl sulphuric acid 1 mol litre⁻¹ to each well. Finally, the optical density at 490 nm was determined using a microtitre plate reader (Vmax kinetic microplate reader, Molecular Devices) [16]. The amount of MtP2 in each region of the six subgroups was compared with

that of control rat brain extracted during pentobarbitone anaesthesia.

Statistical analysis

The results obtained from MtP2 degradation were expressed as a percentage of the control rat brain and represented as a mathematical mean (SD). Physiological variables were analysed using Student's *t* tests between isoflurane and halothane in each group. MtP2 degradation results were analysed using Student's *t* tests or Duncan's multiple comparison, as appropriate, after testing by analysis of variance (ANOVA) within the same region. $P < 0.05$ was considered significant.

RESULTS

Physiological variables (table I). There were no differences in body weight between the groups. There were also no significant differences in physiological variables between isoflurane and halothane before initiation of the ischaemic insult in all groups. However, at 15 min after the initiation of the ischaemic insult, blood glucose concentration with isoflurane anaesthesia in the combined group was significantly ($P < 0.01$) greater than that with halothane anaesthesia. There were no other significant differences in other variables between the isoflurane and halothane groups.

Electroencephalogram (fig. 1). With both isoflurane and halothane anaesthesia, the EEG in the combined group was nearly isoelectric, in contrast to that in the occlusion and hypotension groups, in which electrical activity was well preserved. These results confirmed the successful achievement of forebrain ischaemia, at least in the combined groups, with both anaesthetics [11].

MtP2 degradation (table II)

Frontoparietal cortex. Isoflurane anaesthesia significantly ($P < 0.05$) protected MtP2 from degradation compared with halothane. MtP2 degradation was significantly greater in the combined group with both isoflurane and halothane ($P < 0.001$) compared with the occlusion and hypotension groups.

Brainstem. MtP2 degradation was significantly ($P < 0.05$) greater in the combined group compared with the occlusion group with isoflurane anaesthesia. However, there were no significant differences in MtP2 degradation between isoflurane and halothane in all groups.

Hippocampus. Results were similar to those of the frontoparietal cortex. MtP2 was significantly ($P < 0.01$) less degraded with isoflurane anaesthesia than with halothane in the combined group, and was significantly more degraded compared with the occlusion and hypotension groups with both anaesthetics ($P < 0.001$).

Cerebellum. MtP2 degradation was not found, even in the combined groups, and there was no significant difference between isoflurane and halothane.

DISCUSSION

It has been shown that, in rats, bilateral CCA occlusion combined with a reduction in MAP to 50 mm Hg reduces cerebral blood flow (CBF) to near-zero values in all cerebral cortical areas and in the hippocampus, with a less marked reduction in many subcortical structures and with a comparatively increased flow in the cerebellum [17]. This inter-structural variation may reflect a variable blood supply by the intact vertebral arterial system and collaterals [17]. Judging from the EEG (fig. 1), in the combined groups, at least in the forebrain, CBF was reduced sufficiently to cause the breakdown of cytoskeletal proteins; in the occlusion and hypotension groups, however, it seems to be insufficient to cause breakdown. In this respect, bilateral occlusion of CCA alone has been shown to decrease regional CBF in the forebrain to 50–60% of the normal value (almost the same as the decrease produced by hyperventilation), but results in no notable changes in the energy state of the whole brain tissue [18].

It is not clear how much of a decrease in CBF and

hyperglycaemia accelerates collapse of cytoskeletal proteins during cerebral ischaemia. In this study, during isoflurane anaesthesia, MtP2 in the combined group was significantly less degraded in spite of hyperglycaemia (tables I, II).

The histopathological consequences may be influenced by variations in brain temperature, and measurement of the rectal temperature does not accurately reflect brain temperature during the ischaemic period [21]. However, this is unlikely to have been of significance, as there were no differences in rectal temperature between the isoflurane- and halothane-anaesthetized groups.

It is not known if degradation of cytoskeletal proteins is reversible after re-establishment of the cerebral circulation, or if it extends further during the post-ischaemic period. In addition, it is unclear if cytoskeletal protein breakdown is related to neuronal cell death and if it correlates directly with routine histopathology. Standard histopathology [22] and 2,3,5-triphenyltetrazolium chloride staining [23] have shown isoflurane to have no protective effects compared with halothane.

Isoflurane may be preferable to halothane for patients who are likely to undergo cerebral ischaemia. With isoflurane anaesthesia, the critical CBF which caused EEG ischaemic changes during carotid endarterectomy was significantly less than that with enflurane or halothane anaesthesia, and the incidence of EEG ischaemic changes was significantly less during isoflurane anaesthesia than during either enflurane or halothane anaesthesia [24, 25]. It has been suggested that, if the protective effect of isoflurane depends only on its ability to reduce the cerebral metabolic rate for oxygen ($CMRO_2$) [26], it would not have a protective effect with severe cerebral ischaemia causing electrical silence on the EEG and impairing ion gradients and biosynthesis [27]. In our study, the MtP2 of the frontoparietal cortex and hippocampus in the combined group was less degraded with isoflurane anaesthesia (table II) and is probably independent of its depressive action on $CMRO_2$.

Studies of the mechanism of the collapse of cytoskeletal proteins during cerebral ischaemia have demonstrated that the intracellular Ca^{2+} concentration gradually increases with the passage of ischaemia [28–30]. Increased intracellular Ca^{2+} concentration affects intracellular homeostasis, including activation of protein kinases, phospholipases and

many of the membrane proteins and cytoskeletal proteins [37]. Therefore, in view of the suggested mechanisms of general anaesthesia and the interaction of inhalation anaesthetics with neuronal cell membrane lipids [38], there is a strong possibility that volatile anaesthetics, in some degree, participate in the process of calpain activation, especially in the conversion of the proenzyme to the active form. It seems likely that the mechanism causing the difference in effects on MtP2 degradation between isoflurane and halothane immediately after cerebral ischaemia may depend on the difference in action on the activation mechanism of calpain at the neuronal cell membrane.

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ENFLURANE INCREASES THE ADRENALINE THRESHOLD FOR THE DEVELOPMENT OF SLOW RESPONSES IN ISOLATED CANINE TRABECULAE

S. OSHITA, H. OKA AND T. SAKABE

SUMMARY

To determine if enflurane has different effects on myocardial sensitivity to adrenaline than those previously reported for halothane, we have studied the enflurane–adrenaline interaction in isolated canine trabeculae using doses of adrenaline necessary to produce slow responses (adrenaline threshold for the development of slow responses, ATSR) as an indicator. The preparations were depolarized in Tyrode's solution containing potassium chloride $26 \text{ mmol litre}^{-1}$, then adrenaline concentrations in the solution were increased stepwise. Enflurane 1% had no significant effect, but 2% and 4% significantly increased the ATSR two-fold and seven-fold, respectively. To investigate the influence of enflurane on the adrenaline–adrenoceptor interaction, we studied the effects on the ATSR of 2% enflurane alone or in combination with either prazosin 8 ng ml^{-1} or metoprolol 17 ng ml^{-1} . Compared with the ATSR obtained with 2% enflurane alone, α_1 -block with prazosin did not alter, but β_1 -block with metoprolol significantly increased the ATSR (three-fold). These effects of enflurane are qualitatively as well as quantitatively similar to those reported previously for halothane. Thus, assuming that the MAC value for enflurane is about 2–2.5 times greater than that for halothane, the effects of enflurane on slow channel conductance (antiarrhythmic effects of enflurane) might be about two to three times greater than those of halothane at an equivalent depth of anaesthesia. Such differences may explain in part the clinical observation that ventricular arrhythmias are less likely with enflurane than with halothane. (Br. J. Anaesth. 1993; 71: 253–257).

KEY WORDS

Anaesthetics, volatile: enflurane. Heart: electrocardiogram, arrhythmia. Sympathetic nervous system: catecholamines, adrenaline.

Arrhythmogenic doses of adrenaline differ for different inhalation anaesthetics [1–4]. Enflurane causes fewer arrhythmias than halothane with exogenous administration of adrenaline [1–3, 5, 6]. In addition, enflurane differs from halothane in its propensity to sustain ventricular arrhythmias. Johnston, Eger and Wilson [2] reported that, when ventricular arrhythmias appeared with enflurane, the abnormal beats

were occasional, intermittent and not necessarily sustained. In contrast, when ventricular irritability appeared during halothane anaesthesia, it was often followed by frequent abnormal beats, leading to ventricular tachycardia [2]. Isoflurane is comparable to enflurane in this regard [2–4]. However, with enflurane there is a wide variation in patient response to adrenaline, and the dose–response curve for adrenaline arrhythmias with enflurane is significantly flatter than with halothane or isoflurane [2, 7]. Despite intensive study, the reason for these differences is not clear.

In isolated, partially depolarized guineapig myocardium, Ehara, Hasegawa and Mitsuiye [8] studied the effects of temperature on the myocardial sensitivity to catecholamines using the sensitivity of slow calcium (Ca^{2+}) channels to catecholamine as an indicator. In their experimental model, catecholamine-induced increases in slow channel conductance produced depolarization of the resting membrane in quiescent ventricular muscle. The depolarization was enhanced and often led to automatic activity as temperature decreased. These results suggest that the sensitivity of slow Ca^{2+} channels to catecholamines reflects, at least in part, myocardial sensitivity to catecholamines and that the sensitivity is increased at decreased temperatures. To determine if enflurane has effects on myocardial sensitivity to adrenaline which differ from those reported previously for halothane [9], we also used the sensitivity of slow Ca^{2+} channels to adrenaline—the dose of adrenaline necessary to produce slow responses (adrenaline threshold for the development of slow responses, ATSR)—as an indicator and studied the effects of enflurane, alone or in combination with either selective α_1 - (prazosin) or β_1 - (metoprolol) adrenergic receptor antagonists, on the ATSR in isolated, partially depolarized canine trabeculae.

METHODS

Our study was approved by the Animal Investigation Committee of the hospital. Details of the experimental design were similar to those of our previous

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study [9]. Fourteen unpremedicated mongrel dogs of both sexes, weighing 14–19 kg were anaesthetized with 2% halothane in oxygen. The trachea was intubated and the lungs ventilated via a volume-limited Harvard pump. The beating heart then was removed by sternotomy and placed immediately in oxygenated Tyrode's solution at room temperature. The trabeculae (greater than 10 mm in length and 1–2 mm in diameter) were dissected from the right ventricle and pinned on a small silicon rubber platform. The preparations were stimulated with constant-current pulses introduced at the cut end through small bipolar silver–silver chloride electrodes. The stimulation rate was 0.5 Hz, and the stimuli were either of 1-ms duration and barely greater than threshold (mostly 1 mA) in standard Tyrode's solution (concentrations in mmol litre⁻¹: NaCl, 125; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 1.05; NaHCO₃, 24; NaH₂PO₄, 0.42; glucose, 5.5) or of 10-ms duration and 5 mA in strength in potassium (K⁺)-rich Tyrode's solution containing potassium chloride 26 mmol litre⁻¹. Individual preparations were equilibrated for at least 60 min in standard Tyrode's solution before the start of experiments and then depolarized with K⁺-rich Tyrode's solution to determine ATSR. Standard and K⁺-rich Tyrode's solution were aerated with 95% oxygen–5% carbon dioxide and the pH of both solutions maintained at 7.3–7.4.

Vigorous contractions caused by adrenaline frequently dislodged the intracellular electrode, therefore electrocardiograms were recorded from the surface of the preparations. In our experimental model, increased extracellular resistance was essential to obtain a reliable extracellular voltage decrease along the preparations. For this purpose, the preparations, pinned on a small silicon rubber platform, were surrounded with a uniform and very thin layer of Tyrode's solution [10] and covered by a single layer of cotton mesh. One end of the preparation was placed at the lumen of a 1-mm tube through which oxygenated Tyrode's solution at 37 °C flowed at a rate of 3 ml min⁻¹ maintained constant by a roller perfusion pump. A similar tube was placed at the other end of the preparation and connected to a suction apparatus that was carefully adjusted to provide a thin layer of fluid around the preparations. Two glass microelectrodes filled with KCl 3 mol litre⁻¹ (resistance 10–20 MΩ) were used to record the ECG. The recording electrode was placed 1–1.5 mm from the stimulation electrodes and the reference electrode was placed just beyond the preparation in the fluid stream coming from the perfusion tubing. The extracellular voltage change was recorded by a differential-input, high-impedance amplifier (Nihon Kohden AVM-10) coupled with a cathode follower (Nihon Kohden MEZ-7101) and displayed on a Nihon Kohden VC-10 oscilloscope.

Fast sodium (Na⁺) channels were inactivated by partially depolarizing the preparations in K⁺-rich Tyrode's solution, and slow Ca²⁺ channels were activated by adding adrenaline to the solution. Propagating slow responses were generated when the adrenaline concentrations in the solution reached ATSR. In this study, ATSR was defined as the dose

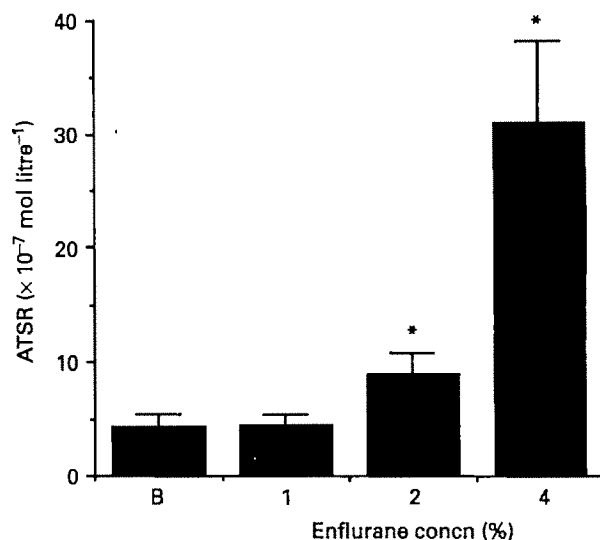


FIG. 1. Adrenaline threshold for the development of slow responses (ATSR) with different concentrations of enflurane (mean, SEM). * Significantly different from baseline (B) ($P < 0.05$).

of adrenaline required to produce nine or more continuous or intermittent slow responses within the last 3 min of a 15-min infusion, which was sufficient time to produce stable effects. If the ATSR was not achieved during 15 min of constant infusion at one concentration of adrenaline, another solution containing a standardized, logarithmically spaced greater dose of adrenaline was infused, and the procedure was repeated until the ATSR was established.

The ATSR was measured before (baseline) and 30 min after administration of each concentration of enflurane (1, 2 and 4%; $n = 7$), and after 2% enflurane alone or in combination with prazosin 8 ng ml⁻¹ or metoprolol 17 ng ml⁻¹ ($n = 7$). Enflurane was equilibrated with Tyrode's solution in the reservoir by passing 95% oxygen–5% carbon dioxide through the calibrated vaporizer for at least 30 min before administration to the preparation. The mean concentrations of 1, 2 and 4% enflurane in solution, verified by gas chromatography (Shimadzu GC-4), were 9.9 (SEM 0.6) mg dl⁻¹, 20.2 (0.1) mg dl⁻¹ and 38.2 (0.9) mg dl⁻¹ ($n = 7$), respectively. The order of administration of drugs was randomized to avoid a possible systematic time-dependent effect.

One-way analyses of variance with critical-difference testing were used to determine statistical significance between baseline values and values obtained at each concentration of each drug. $P < 0.05$ was considered statistically significant.

RESULTS

The mean ATSR values obtained before (baseline) and 30 min after administration of enflurane, alone or in combination with either prazosin or metoprolol, are shown in figures 1 and 2. Enflurane 1% had no significant effect, but 2% and 4% enflurane significantly increased the ATSR (two-fold and seven-fold, respectively) (fig. 1). In combination with 2% enflurane, α_1 -block with prazosin did not alter the ATSR, whereas β_1 -block with metoprolol significantly increased it; compared with the ATSR

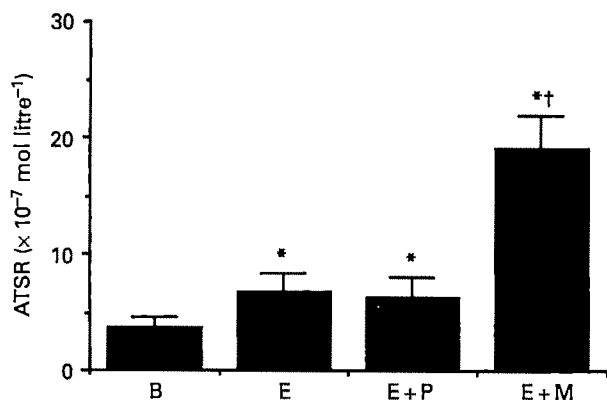


FIG. 2. Adrenaline threshold for the development of slow responses (ATSR) with 2% enflurane alone (E) or in combination with either prazosin 8 ng ml⁻¹ (E+P) or metoprolol 17 ng ml⁻¹ (E+M) (mean, SEM). Significant differences: * from baseline (B); † from the value obtained with 2% enflurane.

obtained with 2% enflurane alone, prazosin 8 ng ml⁻¹ did not change, but metoprolol 17 ng ml⁻¹ significantly increased the ATSR (three-fold) (fig. 2).

DISCUSSION

We studied the direct effects of enflurane on myocardial sensitivity to adrenaline in isolated, partially depolarized canine trabeculae. The myocardial cell, depolarized by K⁺, has a low degree of excitability, one factor responsible for this being an increased membrane shunting (outward K⁺) conductance [11, 12]. That is, in the K⁺-depolarized myocardium, slow inward current is affected by the presence of large outward K⁺ current that flows during the rising phase of the action potential [13]. In most such preparations, muscle excitability is restored or enhanced by addition of catecholamines, which increase slow channel conductance [14], or by addition of small concentrations of barium (Ba²⁺), which reduces membrane shunting conductance [13], to the superfusate—procedures which are thought to intensify the slow inward current.

In the present study, we used the dose of adrenaline required to produce slow responses (the ATSR) as an indicator for myocardial sensitivity to adrenaline. It could be argued that the ATSR might be affected by the changes in the membrane shunting conductance. However, as the increase in membrane shunting conductance is thought to be K⁺-dependent [15], we feel justified in assuming that membrane shunting conductance was kept almost constant throughout our experiments, in which preparations were depolarized equally in K⁺-rich Tyrode's solution containing KCl 26 mmol litre⁻¹ before (when baseline ATSR values were obtained) and during administration of enflurane. In addition, Lynch and colleagues found, in their experiment with isolated, partially depolarized guineapig papillary muscles, that enflurane did not influence K⁺ conductance because it did not change the action potential duration of slow responses. Based on these assumptions, we believe that the ATSR reflects, at least in part, the responsiveness of slow Ca²⁺ channels to adrenaline in isolated heart preparations. As adrena-

line exerts its effects on the heart by increasing slow channel conductance [14], which is triggered by adrenaline-adrenoceptor interaction, the responsiveness of slow Ca²⁺ channels to adrenaline in isolated heart preparations should reflect cardiac responses to adrenaline [8]. Activation of the slow Ca²⁺ channel initiates slow responses, which are responsible for both abnormal automaticity [17] and re-entry [18]. More importantly, a re-entrant mechanism is thought to underlie the arrhythmogenic interaction between volatile anaesthetics and adrenaline [9, 19, 20]. Therefore, we predicted that, at least in part, the threshold for adrenaline-induced arrhythmias and the sensitivity of the myocardium to adrenaline could be investigated using the ATSR as an indicator.

We found that enflurane significantly increased the ATSR in a dose-dependent manner (fig. 1). This action of enflurane, as with that of halothane [9], is closely correlated with its Ca²⁺ entry blocking action. Bosnjak, Supan and Rusch [21] and Eskinder and colleagues [22] recently showed, using the whole-cell voltage-clamp technique, that enflurane reduces the Ca²⁺ current of isolated canine ventricular and Purkinje cells in a concentration-dependent manner. Lynch and colleagues [16] reported that enflurane depresses both the upstroke velocity and the contraction of slow responses in isolated, partially depolarized guineapig papillary muscles. In addition, in our previous study [9], verapamil, in common with enflurane, increased the ATSR in a dose-dependent manner. If these *in vitro* results are applicable to surgical patients, our findings are indicative of an antiarrhythmic action rather than an arrhythmia-producing action of enflurane, and several recent reports suggest that this may be the case. First, ventricular ectopic beats were suppressed during the administration and reappeared after discontinuation of enflurane in a patient undergoing vagotomy and antrectomy [23]. Second, enflurane decreased the incidence of ventricular fibrillation in a canine model of acute occlusion-reperfusion arrhythmias [24]. Third, enflurane decreased the incidence of death from ventricular fibrillation in rats with ligation of the left anterior descending coronary artery [25].

Enflurane has less potential than halothane for production of arrhythmias in the presence of exogenous adrenaline [1–3, 5, 6]. Munson and Tucker [1] reported that, in the dog, the adrenaline dosage for enflurane was 17.1 µg kg⁻¹ and the corresponding value for halothane was 4.6 µg kg⁻¹. Johnston, Eger and Wilson [2] studied patients who were undergoing trans-sphenoidal pituitary operations for which they received nasal and oral submucosal injections of adrenaline to minimize surgical bleeding, and found that the arrhythmic threshold of adrenaline was 10.9 µg kg⁻¹ for enflurane and 2.1 µg kg⁻¹ for halothane, although the dose identified was that necessary to produce a positive response in 50% of the patients (ED₅₀). Sumikawa, Ishizaka and Suzaki [3] reported that, in the dog, the mean values of the arrhythmogenic doses and the corresponding plasma concentrations of adrenaline were 11.43 µg kg⁻¹ min⁻¹ and 206.3 ng ml⁻¹ during enflurane; 2.18 µg

$\text{kg}^{-1} \text{min}^{-1}$ and 38.7 ng ml^{-1} during halothane anaesthesia. These findings indicate that four to five times as much adrenaline is required to produce ventricular arrhythmias during enflurane as during halothane anaesthesia.

If, as postulated in the review of Atlee and Bosnjak [20] and in our previous study [9], inhalation anaesthetics have both antiarrhythmic (Ca^{2+} entry blocking actions) and arrhythmia-producing effects (effects on the refractory period, Na^+ channel conductance and passive membrane properties), such differences between enflurane and halothane in the arrhythmogenic doses of adrenaline might exist because the antiarrhythmic effects of enflurane are greater or its arrhythmogenic effects are smaller (or both) than those of halothane. We have demonstrated in the present study that the effects of enflurane on the ATSR are similar to those reported previously for halothane in the same concentration: 2% and 4% enflurane increased the ATSR two-fold and seven-fold, while 2% and 4% halothane increased it two-fold and five-fold, respectively [9]. Thus, assuming that the MAC value for enflurane is about 2–2.5 times that of halothane [26], our results suggest that the antiarrhythmic effects of enflurane may be about 2–3 times greater than those of halothane at the equivalent depth of anaesthesia. Bosnjak, Supan and Rusch [21] and Eskinder and colleagues [22] reported recently on whole-cell voltage-clamp experiments with single isolated canine ventricular or Purkinje cells in which, quantitatively, enflurane and halothane produce a similar inhibition of Ca^{2+} channel currents at equianaesthetic concentrations. These results seem to conflict with our data, but it should be noted that the aim of their studies differed from ours. They investigated the relationship between the negative inotropic and chronotropic actions of volatile anaesthetics and inhibition of Ca^{2+} channel currents, and so they needed to record isolated Ca^{2+} channel current. For this purpose (in order to eliminate Na^+ and K^+ currents) they substituted Ba^{2+} , tetraethylammonium chloride and caesium ion for Ca^{2+} , Na^+ and K^+ . In contrast, we aimed to investigate the direct effects of enflurane on the arrhythmia-producing actions of adrenaline in isolated heart preparations and therefore we induced slow responses by adding adrenaline to the superfusate containing Ca^{2+} , Na^+ and K^+ . Slow inward current is carried by Na^+ and by Ca^{2+} [27], and outward K^+ current may flow through slow channels [27, 28]. Therefore, the differences between the results reported by Bosnjak, Supan and Rusch [21] and Eskinder and colleagues [22] and those of our present study might be attributable to the differences between isolated Ca^{2+} current and slow inward current. If, as described above, slow responses are responsible for re-entry type arrhythmias [18], which are thought to underlie the arrhythmogenic interaction between volatile anaesthetics and adrenaline [9, 19, 20], it should be possible to investigate the effects of enflurane on myocardial sensitivity to adrenaline (antiarrhythmic effects of enflurane in the present study) using the ATSR as an indicator. In contrast, Atlee and Rusy [29, 30] and Atlee and Alexander [19] have shown that halothane

prolongs A–V nodal, His–Purkinje and ventricular conduction, whereas enflurane prolongs only A–V nodal conduction in dogs. From these results, they concluded that conduction changes such as occur with halothane are necessary for ventricular arrhythmias caused by re-entry of excitation and that enflurane is less likely to cause these arrhythmias. In addition, Burt and Spray [31] studied the effects of halothane and enflurane on the passive membrane properties of the myocardium (gap junction-mediated intercellular communication) in cultured neonatal rat cardiac myocytes, and found that complete block of junctional conductance occurred with smaller concentrations of halothane than enflurane.

To investigate the influence of enflurane on adrenaline–adrenoceptor interaction, we studied the effects on the ATSR of 2% enflurane alone or in combination with prazosin or metoprolol. In our study of the effects of halothane on adrenaline–adrenoceptor interaction [9], we reported that α_1 -block with prazosin 4, 8 and 16 ng ml^{-1} or droperidol 20, 40 and 80 ng ml^{-1} did not alter the ATSR, whereas β_1 -block with metoprolol 8, 17 and 34 ng ml^{-1} significantly increased the ATSR (two-fold, four-fold and six-fold, respectively). In the same study [9], we reported also that the effects of prazosin and metoprolol on the ATSR were not influenced by halothane: compared with the ATSR obtained with 2% halothane, prazosin 8 ng ml^{-1} did not alter the ATSR, whereas metoprolol 17 ng ml^{-1} significantly increased the ATSR (five-fold). Similar to the results reported for halothane [9], the effects of prazosin and metoprolol when given in combination with 2% enflurane were almost the same as those obtained for each drug given alone: compared with the ATSR obtained with 2% enflurane alone, prazosin 8 ng ml^{-1} did not change the ATSR, but metoprolol 17 ng ml^{-1} significantly increased the ATSR (three-fold) (fig. 2). These results suggest that the influence of enflurane on adrenaline–adrenoceptor interaction is qualitatively the same as that of halothane [9].

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EFFECT OF DIABETES MELLITUS ON THE CARDIOVASCULAR RESPONSES TO INDUCTION OF ANAESTHESIA AND TRACHEAL INTUBATION

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SUMMARY

We have compared cardiovascular responses to induction of anaesthesia and to tracheal intubation after propofol 2.5 mg kg^{-1} and pancuronium 0.1 mg kg^{-1} in 10 diabetic and 10 matched, non-diabetic (control) ASA I patients. Anaesthesia was maintained with 0.8% enflurane and 50% nitrous oxide in oxygen, with assisted ventilation. The trachea was intubated 3 min after induction of anaesthesia. All 10 diabetic patients (but no controls) had abnormal autonomic function when tested on the day before surgery. There was no difference between the two groups in the pre-induction cardiovascular state. Mean arterial pressure and vascular resistance decreased after induction in each group ($P < 0.05$). Heart rate increased ($P < 0.01$) and cardiac index was sustained in the control group, but in the diabetic group heart rate did not change and cardiac index decreased ($P < 0.01$). There was an earlier decrease in stroke index in the diabetic group (2 min) compared with the controls (5 min). After tracheal intubation, heart rate and cardiac index in the control group and cardiac index in the diabetic group remained unchanged. However, there was a greater increase in heart rate, mean arterial pressure and vascular resistance in the diabetic group compared with the controls after tracheal intubation ($P < 0.05$). The exaggerated pressor response to tracheal intubation, in the diabetic patients, may reflect autonomic dysfunction. (Br. J. Anaesth. 1993; 71: 258–261)

KEY WORDS

Anaesthetics, intravenous: propofol. Complications: diabetes, neuropathy. Intubation: complications.

Several studies have shown increased surgical morbidity and mortality in patients with diabetes mellitus [1–4], thought to be caused by the systemic dysfunction associated with this disease [2, 5]. Autonomic neuropathy, which can occur in both insulin-dependent and non-insulin-dependent diabetic patients [6], may potentially modify the normal cardiovascular responses during anaesthesia and render the diabetic patient more susceptible to cardiovascular morbidity. Although autonomic

neuropathy has been associated with cardio-respiratory arrest in the perioperative period [1], its influence on cardiovascular responses during anaesthesia has not been studied. The present study was designed to compare diabetic with non-diabetic patients in their changes in mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), stroke volume (SV) and vascular resistance (SVR) in response to induction of anaesthesia and tracheal intubation after propofol and pancuronium.

PATIENTS AND METHODS

After receiving local Ethics Committee approval, we obtained informed consent from 10 diabetic patients (presenting consecutively) and 10 ASA I patients (matched for sex, age, height and weight) undergoing vitreous surgery. Only patients without overt cardiovascular disease were studied. In an attempt to standardize laryngoscopy, any patient who was known or found to present difficulty in tracheal intubation was excluded. Eight patients were insulin-dependent and two were non-insulin-dependent diabetics. Eight of the 10 diabetic patients were undergoing vitreous surgery for diabetic eye disease.

Three standard cardiovascular autonomic function tests were performed on the day before surgery [7]:

30:15 R-R ratio—the patient's heart rate was monitored whilst they changed from a supine to an erect position, the 30th and 15th R-R intervals identified and the ratio of the two derived.

Maximum and minimum heart rate difference during three deep breaths cycle.

Decrease in systolic arterial pressure during postural change.

On arrival of the patient in the anaesthetic room, a 20-gauge cannula was inserted into a vein in the dorsum of the patient's right hand. As all the

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diabetic patients were first on the operating list, it was standard practice to omit the insulin or oral hypoglycaemic drugs on that morning, and to ensure against hypoglycaemia or hyperglycaemia (blood sugar 9–15 mmol litre⁻¹). Arterial pressure was measured using an automated non-invasive method (Dinamap) timed to cycle at 1-min intervals.

After skin preparation, current sensing, current source and ECG sensing electrodes (Medicost-VL-OO-S) were placed on the patient according to manufacturer's instructions and attached to a trans-thoracic bioimpedance continuous cardiac output monitor (BoMed NCCOM3, Software Version 7, BoMed Biomedical, Irvine, CA). This monitor was used to measure and derive continuously HR, SV and CO, updated at every 12th heart beat. A period of 5 min was allowed for the patient's recordings to stabilize. Preinduction measurements of MAP, HR, CO and SV were then recorded.

All patients were premedicated with temazepam 20 mg given 2 h before operation. Anaesthesia was induced with propofol 2.5 mg kg⁻¹ given over 30 s, followed immediately by pancuronium 0.1 mg kg⁻¹. Any pain on injection of propofol was recorded. The same anaesthetist intubated the trachea of each patient without difficulty 3 min after induction of anaesthesia. Anaesthesia was maintained with 0.8% enflurane and 50% nitrous oxide in oxygen, with a fresh gas flow of 100 mg kg⁻¹. Ventilation was assisted with mask and Bain circuit before tracheal intubation and then with the Manley MP2 ventilator (tidal volume 10 ml kg⁻¹).

For analysis, post-induction measurements of HR, MAP, CO and SV were recorded at 1-min intervals for the 3 min between induction and tracheal intubation and then for 5 min after intubation. SVR was derived using following formula:

$$SVR = \frac{MAP \times 79.9}{CO}$$

Each patient's body surface area was derived from their height and weight; cardiac index (CI) and stroke index (SI) were calculated. Statistical analysis was performed using paired and unpaired *t* tests with Bonferroni correction and analysis of variance (ANOVA). *P* values were two-tailed; *P* < 0.05 was considered significant.

RESULTS

There were no significant differences in age, height or weight between the diabetic and non-diabetic control groups (table I). No patient complained of pain during induction of anaesthesia and none coughed during tracheal intubation. None of the patients required i.v. glucose before anaesthesia.

Autonomic function

All patients in the control group had normal autonomic function before operation. At least one autonomic function test was abnormal in each of the diabetic patients (table II). The two heart rate tests were found to be abnormal in all 10 diabetic patients; arterial pressure was abnormal in one and borderline abnormal in two of them.

Cardiovascular variables

Heart rate. There was no significant difference between the two groups in mean preinduction HR (fig. 1). In the control group (but not in the diabetic group) the HR increased after induction of anaesthesia (*P* < 0.01) and did not increase further after tracheal intubation (fig. 1). In the diabetic group, HR increased significantly only in response to tracheal intubation (*P* < 0.01). Mean HR was greater in the control group compared with the diabetic group during the study (ANOVA, *P* < 0.01) (fig. 1).

Cardiac index. There was no significant difference between the two groups in mean preinduction CI (fig. 1). In the diabetic group, CI decreased to less than preinduction 2 min after induction of anaesthesia (*P* < 0.01), but it did not change significantly after tracheal intubation. In the control group, CI was maintained after induction of anaesthesia and decreased gradually after tracheal intubation to reach a value significantly less than preinduction, 3 min after intubation (*P* < 0.05). Mean CI was greater in the control group compared with the diabetic group (ANOVA, *P* < 0.05) (fig. 1).

Stroke index. In the diabetic group, mean SI decreased 2 min after induction (*P* < 0.01) and

TABLE I. Patient characteristics (mean (range or SD)). BSA = Body surface area

Group	Age (yr)	Height (cm)	Weight (kg)	BSA (m ²)
Diabetic	44 (27–68)	169 (7.7)	76 (12.6)	1.85 (0.16)
Control	44 (28–65)	171 (6.2)	69 (7.1)	1.79 (0.12)

TABLE II. Results of the autonomic function tests in each of the diabetic and control patients (actual value (category)). N = Normal (30:15 ≥ 1.04; HR diff. ≥ 15 beat min⁻¹; decrease in AP ≤ 10 mm Hg); B = borderline (30:15 = 1.01–1.03; HR diff. = 11–14 beat min⁻¹; decrease in AP = 11–29 mm Hg); A = abnormal (30:15 ≤ 1; HR diff. ≤ 10 beat min⁻¹; decrease in AP ≥ 30 mm Hg)

Patient No.	30:15 R-R ratio	Max-min HR diff. (beat min ⁻¹)	Decrease in AP (mm Hg)
Diabetics			
1	1 (A)	7 (A)	12 (B)
2	1 (A)	0 (A)	28 (B)
3	1.2 (N)	6 (A)	10 (N)
4	1 (A)	6 (A)	6 (N)
5	1 (A)	0 (A)	30 (A)
6	1 (A)	0 (A)	8 (N)
7	1 (A)	0 (A)	10 (N)
8	1 (A)	3 (A)	5 (N)
9	0.7 (A)	0 (A)	10 (N)
10	1 (A)	0 (A)	8 (N)
Controls			
1	1.3 (N)	25 (N)	0 (N)
2	1.01 (N)	34 (N)	0 (N)
3	1.04 (N)	15 (N)	8 (N)
4	1.1 (N)	15 (N)	0 (N)
5	1.04 (N)	28 (N)	2 (N)
6	1.1 (N)	21 (N)	2 (N)
7	1.05 (N)	21 (N)	0 (N)
8	1.18 (N)	15 (N)	5 (N)
9	1.04 (N)	15 (N)	5 (N)
10	1.05 (N)	28 (N)	0 (N)

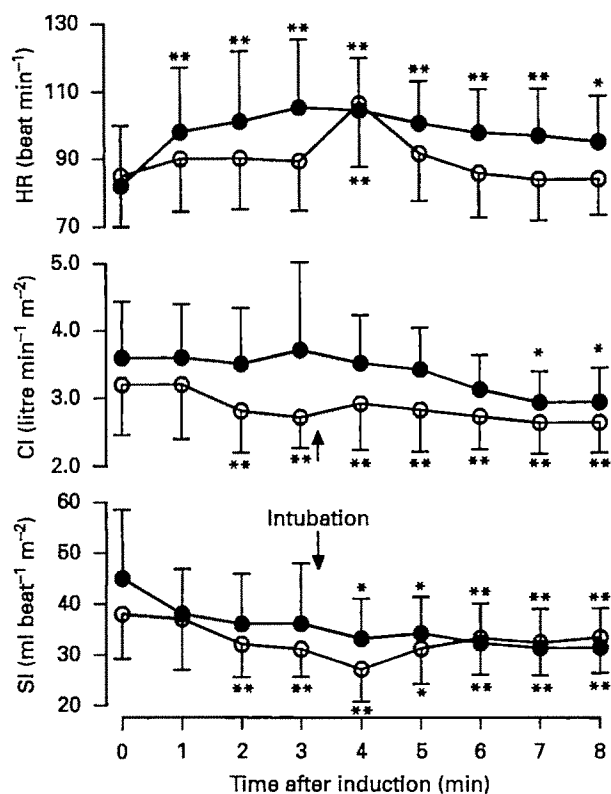


FIG. 1. Changes in heart rate (HR), cardiac index (CI) and stroke index (SI) in diabetic (○) and non-diabetic (●) patients after induction of anaesthesia and tracheal intubation (mean, SD). Significant difference from preinduction value within group: * $P < 0.05$; ** $P < 0.01$.

decreased again 1 min after intubation ($P < 0.01$) (fig. 1). In the control group, mean SI did not decrease to significantly less than its mean value before induction until 1 min after intubation ($P < 0.05$).

Mean arterial pressure. Induction of anaesthesia was associated with a significant decrease in MAP in each group ($P < 0.05$) (fig. 2). After tracheal intubation there was a greater increase in the diabetic patients than in the control group. Thus in the diabetic group MAP was significantly greater than before induction at 1 min after tracheal intubation ($P < 0.05$), while in the control group MAP after intubation was similar to that before induction (fig. 2).

Vascular resistance. SVR decreased in each group after induction of anaesthesia ($P < 0.05$ control; $P < 0.01$ diabetic). After tracheal intubation, there was a greater increase in SVR in the diabetic group compared with the controls ($P < 0.05$), that in the diabetic group alone being significantly greater than preinduction ($P < 0.05$) (fig. 2).

DISCUSSION

Sullivan and colleagues [8] found that, during anaesthesia, cardiac output derived by the trans-thoracic bioimpedance monitor correlated well with that derived using the thermodilution technique and others have shown good correlation in other situations [9, 10]. The cardiovascular variables before induction were found to be similar in the

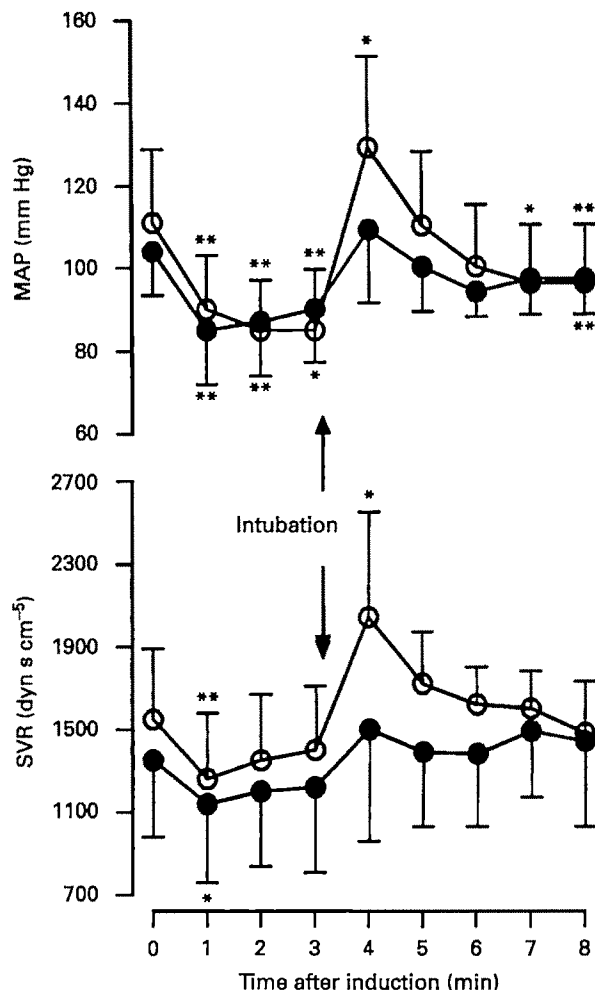


FIG. 2. Changes in mean arterial pressure (MAP) and systemic vascular resistance (SVR) in diabetic (○) and non-diabetic (●) patients after induction of anaesthesia and tracheal intubation (mean, SD). Significant difference from preinduction value within group: * $P < 0.05$; ** $P < 0.01$.

diabetic and control patients, but their responses to induction of anaesthesia and tracheal intubation differed significantly. Both the parasympathetic and the sympathetic systems play a part in the reflexes associated with the three autonomic function tests performed in this study. However, abnormalities in the two heart rate tests are predominantly associated with parasympathetic dysfunction and abnormalities in the arterial pressure test are caused predominantly by sympathetic dysfunction [11, 12]. In this study, the sympathetic function test was not normal in three and the parasympathetic function tests were abnormal in all 10 diabetic patients studied, and this may be the cause of the altered haemodynamic responses in the diabetic group.

In a British community survey, Neil and colleagues [13] found a single abnormal cardiovascular response in 16% of diabetic patients. Ziegler and colleagues [6] found that autonomic dysfunction occurred within 2 years of onset of diabetes. Our findings suggest that all diabetic patients presenting for vitreous surgery may have autonomic dysfunction.

In the control group, the administration of pancuronium was associated with the expected

tachycardia. However, this response was absent in the diabetic group. Lee Son and Waud [14] and, Salt, Barnes and Conway [15] have shown that pancuronium may cause tachycardia by blocking postjunctional muscarinic receptors on the sinoatrial node and prejunctional muscarinic receptors on cardiac sympathetic nerves (both innervated normally by the vagus nerve). We found that the parasympathetic function tests were abnormal in the diabetic group and this abnormality may be the basis of the altered response to pancuronium in these patients. This suggests that, in diabetic subjects, the heart rate response to atropine and neostigmine given normally during antagonism of neuromuscular block may be abnormal.

CI did not decrease in the control group after induction of anaesthesia, because of the pancuronium-induced tachycardia. However, in the diabetic group, the increase in HR did not occur and CI decreased. CI was therefore smaller in the diabetic group compared with controls. The decrease in SI may be attributed to the cardiac effects of propofol [16]. Coleman and colleagues [17] have shown that an increase in cardiac output after administration of pancuronium is caused by the increase in HR and not any effect on stroke volume. Our findings support this. The changes in MAP were a reflection of those occurring in SVR. We have found this in a previous study [16]. There was a greater increase in MAP, HR, and SVR after tracheal intubation in the diabetic patients compared with the controls. During laryngoscopy and tracheal intubation, sensations from the pharynx and trachea are transmitted centrally via the vagus nerve and cause reflex stimulation of the sympathetic and sympatho-adrenal systems. This results in release of both adrenaline and noradrenaline [18, 19]. Cummings, Russell and Frewin [20] showed that, although pancuronium inhibits noradrenaline reuptake, it does not affect the pressor response to tracheal intubation. In the presence of autonomic dysfunction, one might have expected an obtunded pressor response to tracheal intubation. The diabetic patients were mainly in the normal to borderline section when tested for sympathetic dysfunction. It

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A NEW METHOD OF MEASURING CARDIAC OUTPUT IN MAN USING LITHIUM DILUTION

R. A. F. LINTON, D. M. BAND AND K. M. HAIRE

SUMMARY

We describe a new indicator dilution method of measuring cardiac output in man. A bolus injection of lithium chloride 0.6 mmol was given via a central venous catheter and arterial plasma $[Li^+]$ recorded using a specially developed sensor incorporating an Li^+ -selective electrode. Cardiac output was derived from the lithium dilution curve, with a correction for packed cell volume. Lithium dilution cardiac output (LiDCO) was compared with thermodilution cardiac output (TD) using 22 lithium sensors in nine patients. For each sensor, one LiDCO was measured immediately before and one immediately after three TD estimations and mean values of LiDCO and TD derived. The correlation coefficient, r , was 0.89; slope of the regression 0.84; y intercept 0.72; bias 0.3 (0.5) litre min^{-1} (mean (TD-LiDCO) (1 SD). LiDCO appeared to be a safe, simple and accurate technique which does not require insertion of a pulmonary artery catheter. (Br. J. Anaesth. 1993; 71: 262–266)

KEY WORDS

Heart: cardiac output. Measurement techniques: cardiac output, thermodilution.

Existing methods of cardiac output measurement have several disadvantages. Both the indicator dilution method using indocyanine green and the Fick method can be accurate but they are difficult and impractical. Non-invasive methods such as Doppler and thoracic bioimpedance are inaccurate [1–4] and the most commonly used thermodilution [5] requires a pulmonary artery catheter with its attendant risks. In the words of a *Lancet* editorial "...development of a simple, safe, reliable and cheap method remains a worthwhile objective" [6].

We have developed a new indicator dilution method which is minimally invasive, requiring only central venous and arterial catheters which would probably already have been inserted in patients whose management would be helped by cardiac output measurement. The indicator is lithium (Li^+) which is rapidly injected into a central vein (as lithium chloride ($LiCl$) 0.6 mmol in 2 ml) and detected using a lithium-selective electrode in arterial blood.

PATIENTS AND METHODS

We studied nine patients during the immediate postoperative period after either coronary artery bypass grafting (seven patients) or aortic valve replacement (two patients). The study was approved by the Ethics Committee and written, informed consent was obtained from all patients. The patients' ages and weights were in the ranges 38–73 yr and 64–90 kg and none was receiving oral lithium.

After induction of anaesthesia, a thermodilution pulmonary artery catheter (Baxter Edwards Swan-Ganz) was inserted via the right internal jugular vein. The catheter was advanced until the pressure recorded from the injectate port showed this to be in the right ventricle. The catheter was then withdrawn 2 cm further than the point at which the pressure changed from ventricular to atrial. An arterial cannula (22-, 20- or 18-gauge, depending on the normal practice of the anaesthetist) was inserted into a radial or brachial artery. During the study, which took less than 1 h, the patients were sedated with morphine and propofol infusions and their lungs ventilated using a Servo ventilator.

Thermodilution cardiac output

TD measurements were carried out using a Baxter (Edwards COM-2) thermodilution computer. Injections of ice-cold 5% glucose 10 ml were made into the right atrium and a minimum of four readings was made as rapidly as possible. The first was rejected, as were any which differed by more than 0.5 litre min^{-1} from the mean of the others. In this case, further readings were taken until three acceptable values were obtained. TD was taken as the mean of these values. No individual value differed from the mean of that set by more than 10.9%.

Lithium dilution cardiac output

The lithium sensor (fig. 1) consisted of a 2–3 cm length of 18-gauge Teflon tubing containing an internal reference material and with a PVC membrane dip-cast over one end. The membrane contained the crown ether lithium ionophore VI (6,6-Dibenzyl-14-crown-4; 6,6-Dibenzyl-1,4,8-11 tetra-

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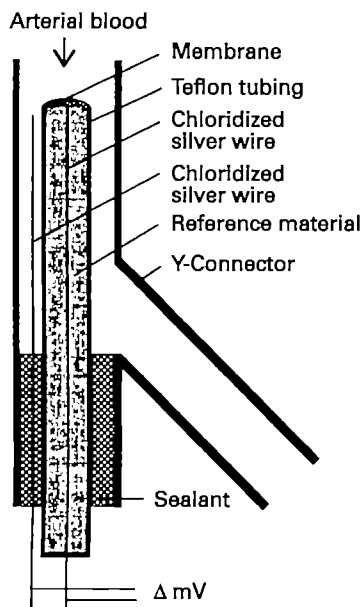


FIG. 1. Diagram of the lithium sensor.

oxacyclotetradecane) (Fluka) which made it selectively permeable to Li^+ [7]. The sensor was mounted in a Y-connector (Vygon, 889). The voltage across the membrane was recorded between two chloridized silver wires, one in the reference material and the other in the lumen of the Y-connector. This second wire was coated with polyurethane (Tecoflex) to protect the silver chloride from plasma proteins. The voltage was recorded via an optically isolated preamplifier [8], displayed on a chart recorder (PAR 1000) and recorded on magnetic tape for later analysis.

Before the sensors were mounted in the Y-connectors, they were tested *in vitro* to ensure that the voltages recorded in saline and $[\text{Li}^+]$ $1 \text{ mmol litre}^{-1}$ in saline were correct: change from one solution to the other should produce a 10-mV change in membrane potential. The sensor was then sealed into one limb of the Y-connector and sterilized by immersion in glutaraldehyde. The glutaraldehyde was washed out with saline and the calibration repeated. The Y-connector with three-way tap was connected via a second three-way tap to the hub of the arterial cannula. A 10-cm length of tubing and 20-ml syringe were connected to the open limb of the Y-connector so that blood could flow past the sensor into the syringe. The flow varied depending on the size of the arterial cannula, packed cell volume (PCV) and arterial pressure, but was in the range $15\text{--}30 \text{ ml min}^{-1}$; at no stage did blood or flushing fluid pass back over the sensor into the arterial catheter. Blood was allowed to flow past the sensor until a stable baseline voltage was recorded and then LiCl 0.6 mmol (2 ml of a $0.3\text{-mol litre}^{-1}$ solution) was injected into the superior vena cava via one of the catheters in the right internal jugular vein. The deadspace of this catheter had previously been cleared with the LiCl solution and care was taken to inject exactly 2 ml. Ampoules containing 10 ml of LiCl $0.3 \text{ mol litre}^{-1}$ had been prepared by St Thomas's Hospital pharmacy. When the second LiDCO curve had been obtained, another sensor was

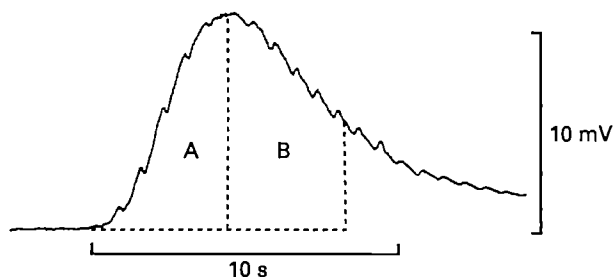


FIG. 2. A LiDCO curve showing its division into areas A and B. The total area which would have been inscribed by the curve had there been no recirculation = $(A + B + B/2)$.

attached and the same procedure repeated. Each lithium sensor was used to make two cardiac output measurements. Between these two measurements, three TD measurements were made. The LiDCO and TD estimates were therefore not simultaneous, but the intervals between them were as short as possible, usually less than 1 min. The mean of the LiDCO values was compared with the mean of the TD values. Up to three sensors were tested in this way in each patient; results were obtained from 22 electrodes in the nine patients.

The plasma flow was derived from the dilution curve by dividing the injected dose of LiCl by the area which would have been inscribed by the curve if there had been no recirculation of the Li^+ . Using an iterative model based on a two-compartment filter, a simple algorithm was developed to allow calculation of this area (fig. 2): it is equal to $A + B + B/2$, where A = the area enclosed between the start of the increase in $[\text{Li}^+]$ and the peak concentration and B = the area enclosed between the peak concentration and a point on the curve when $[\text{Li}^+]$ had decreased to 50% of its peak. This procedure is similar to that used for thermodilution, except that the cut-off is slightly earlier, to avoid recirculation. On the basis of this model, the calculated total area was correct to within 2%. The areas were measured by amplifying the curves during playback on to standard paper, cutting out the areas A and B, weighing them and multiplying these weights by the area per unit weight of the paper. Weighing was accurate to $\pm 0.5 \text{ mg}$ using a Sartorius balance, which is equivalent (in terms of cardiac output) to an accuracy of $\pm 0.5\%$. The area is in units of $\text{mmol litre}^{-1} \text{ s}$. Cardiac output was then derived:

Cardiac output (litre min^{-1}) =

$$\frac{\text{dose of LiCl (mmol)} \times 60}{\text{area of curve (mmol litre}^{-1} \text{ s)} \times (1\text{-PCV})}$$

where the factor 60 converts flow from litre s^{-1} to litre min^{-1} and division by (1-PCV) converts plasma flow to blood flow, since Li^+ is distributed only in the plasma fraction of blood. PCV was calculated as haemoglobin concentration (g dl^{-1}) divided by 33 (Radiometer OSM3 Hemoximeter).

The sensor gave a constant reading in blood in the absence of any Li^+ , as a result of the cross-sensitivity of the ligand with Na^+ , so that it read the background plasma $[\text{Na}^+]$ ($140 \text{ mmol litre}^{-1}$) as approximately $[\text{Li}^+]$ $2.4 \text{ mmol litre}^{-1}$. The injections of LiCl

0.6 mmol produced small increments on top of this background voltage and, over this narrow range, the voltage change of the sensor approximated closely to a linear response to $[\text{Li}^+]$. The linearity of the response over this range was confirmed by adding aliquots of Li^+ to blood *in vitro*. LiDCO values could therefore be calculated without transformation of the curves before determining their areas.

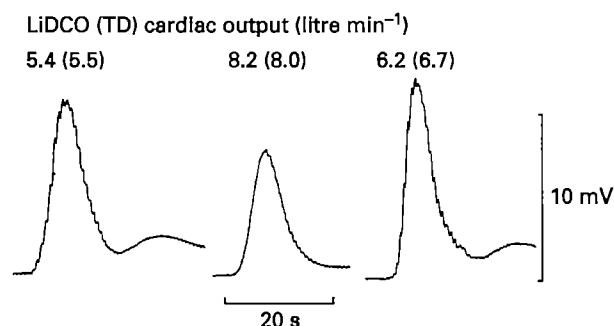


FIG. 3. Examples of LiDCO curves obtained in different patients. The cardiac output is shown above each curve with the corresponding TD reading in brackets. From left to right, the areas of the curves are 9.08, 6.07 and 8.49 mmol litre⁻¹ s, with corresponding haemoglobin concentrations of 8.9, 9.1 and 10.4 g dl⁻¹.

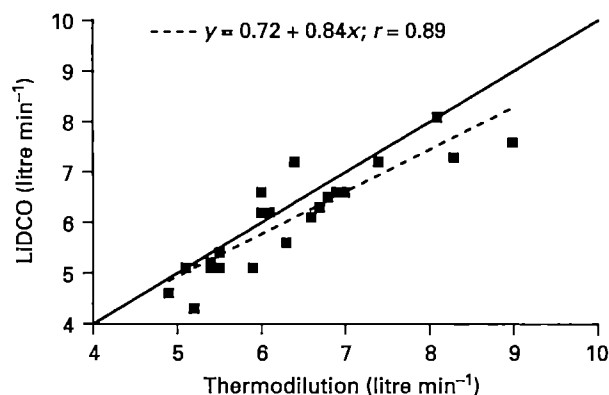


FIG. 4. Correlation between LiDCO and TD for 22 lithium sensors. Each point represents the mean of two LiDCO values plotted against the mean of three TD values. The solid line represents $y = x$ and the dotted line the least squares regression.

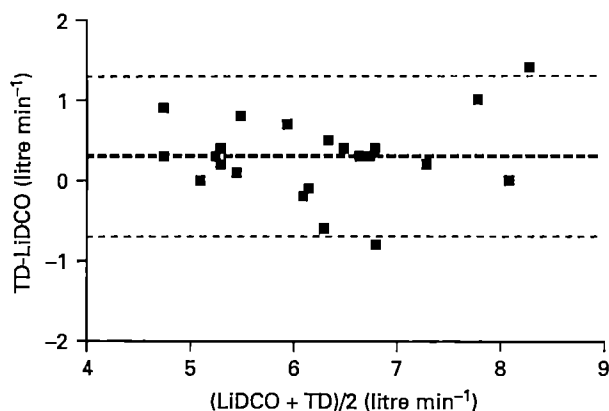


FIG. 5. Graph of the differences between LiDCO and TD plotted against the mean. The thick dotted line shows the bias (295 ml min⁻¹) and the thin dotted lines show the ± 2 SD limits.

RESULTS

Figure 3 shows three LiDCO curves obtained in different patients at different cardiac outputs. Peak plasma $[\text{Li}^+]$ reached in the third curve was about 1.3 mmol litre⁻¹. Results were obtained from 22 of 27 electrodes in the nine patients. Results from five electrodes were rejected either because of excessive drift or because they had a reduced span when calibrated after use. The correlation between the LiDCO and TD measurements is shown in figure 4. The average of all the LiDCO readings was 6.1 litre min⁻¹ and that of the TD readings 6.4 litre min⁻¹; the average TD estimate was 295 ml min⁻¹ greater than the LiDCO. This is also shown in figure 5, in which bias $(\text{TD} - \text{LiDCO})$ is plotted against $(\text{LiDCO} + \text{TD})/2$, as recommended by Bland and Altman [9]. The SD of the bias was 498 ml min⁻¹.

DISCUSSION

It is not possible to conclude from our data that LiDCO is more accurate than TD, although this may prove to be the case. Stetz and colleagues [10] analysed 14 reports of the reliability of TD and concluded that, for commercially available systems, there should be a minimum difference of 13% between triplicate (or 22% between single) measurements for it to be considered to be clinically significant. If this magnitude of error existed in the TD estimations in the present study, this could have accounted for a significant proportion of the discrepancy between the two methods. There are circumstances, such as tricuspid regurgitation or peripheral migration of the Swan-Ganz catheter, in which TD has been shown to give unreliable measurements [11, 12].

Lithium was the obvious choice of cation as the indicator in this technique as various ligands are available for the construction of Li^+ -selective electrodes and Li^+ is not normally present in the blood, except in trace quantities, nor is it bound by protein. The electrodes have a fast frequency response and, as they respond to proportional changes in concentration, large signals are produced for small changes close to zero, in accordance with the Nernst equation. The main shortcomings of ion-selective electrodes, namely long-term drift and the difficulty of absolute calibration, are of no significance because, in the computation, it is the area under each curve which is used and this is inscribed within 15 s.

There were several possible problems with this technique. One was that lithium might be taken up by the blood cells or lungs during its transit from the right atrium to peripheral arterial blood; either of these effects would result in a falsely increased estimate of the cardiac output. The present study indicates that such uptake is unlikely to be significant. The addition of aliquots of lithium chloride to whole blood *in vitro* showed that there was no measurable reduction in plasma $[\text{Li}^+]$ during at least the first 30 min after the additions. The TD estimate of cardiac output was, on average, 295 ml min⁻¹ greater than the LiDCO estimate. Although this difference is not significant, had there been a

significant loss of Li^+ there would have been an obvious difference in the opposite direction.

Another possible problem was that the sudden transition from room temperature saline to warm blood may have caused an unacceptable drift in electrode potential, or the non-heparinized blood may have caused a rapid deterioration in electrode span. In practice, five of the 27 sensors used in this investigation were rejected because of either drift or failure to calibrate; it is likely that these failures were caused by errors in the construction of the sensors, rather than a fault in the fundamental design.

Another problem could have been that the frequency response of the system, with its deadspace of taps and associated tubing, might have been inadequate for the purpose. The dilution curves themselves contain internal evidence that this is not a limiting feature. The cardiac oscillations in $[\text{Li}^+]$ shown in figure 2 and the first and third curves in figure 3 are genuine rather than pressure or flow artefacts, as they do not appear on the trace before the increase in $[\text{Li}^+]$ or during the recirculation peak. They are caused when $[\text{Li}^+]$ is increasing or decreasing rapidly and occur because the first fraction of blood to fill the left ventricle is the last to be ejected. Whether or not cardiac oscillations are seen depends on the response time of the sensing system, the rate at which $[\text{Li}^+]$ is changing in pulmonary venous blood, left ventricular ejection fraction and heart rate. The present flow-through cells were relatively crude and based upon available components. A considerable reduction in deadspace is possible and a total blood sample of less than 5 ml per measurement is our objective.

Measurement of cardiac output by lithium dilution has potential advantages over other methods. It is accurate, quick, simple and avoids the hazards of pulmonary artery catheterization. Non-invasive methods of measuring cardiac output, such as thoracic bioimpedance and Doppler ultrasound, have the advantage of continuous monitoring but are both difficult and inaccurate, especially in patients who have recently undergone heart or aortic surgery.

Lithium is distributed initially into plasma and then extracellular fluid and later into a volume equal to that of total body water (40 litre in a 70-kg subject). It is not metabolized and is excreted almost entirely in the urine, with a renal clearance of approximately 25% of glomerular filtration rate or about 30 ml min^{-1} [13]. The therapeutic range is $0.4\text{--}0.8 \text{ mmol litre}^{-1}$, with the blood being taken 12 h after the last dose [14]; peak concentrations may be considerably greater than those at 12 h [15]. The acute lethal dose of lithium varies, but is generally associated with a plasma concentration greater than $3.5 \text{ mmol litre}^{-1}$ 12 h after ingestion. The greatest concentration in our patients was about $1.3 \text{ mmol litre}^{-1}$, but this was only for a few seconds and would not have been transmitted to the extracellular fluid. We have found that awake subjects experience no sensation of any sort when given doses of LiCl up to 1 mmol, rapidly i.v. A recommendation would need to be made concerning the total number of LiDCO measurements which could be made safely within any period. We are not yet in a position to do so, but

the number would be large before the therapeutic concentration of lithium in extracellular fluid was reached, even in the absence of excretion. The dose used (0.6 mmol) was chosen to give an adequate signal over a range of cardiac outputs. It may be possible to develop a membrane which has a greater selectivity ratio for Li^+ over Na^+ and this would allow a corresponding reduction in the dose of lithium. LiDCO would probably not be a suitable method for patients receiving oral lithium.

Improved quality control in manufacture of the electrodes should avoid any failures and they will be disposable, sterilized by gamma irradiation and stored dry. The signal from the lithium sensor would, of course, be analysed by computer at the bedside, with immediate display of the curve and calculated cardiac output. It is possible that a scaled-down version of the sensing system would allow cardiac output measurements in babies and small children, with correspondingly smaller doses of LiCl .

With further development, we hope that lithium dilution may prove to be an accurate method of measuring cardiac output, while avoiding the hazards associated with pulmonary artery catheters. It would allow measurements to be made within a few minutes in patients in whom central venous and arterial catheters are in place, without exposing them to any further risk. (The sensor is the subject of patent applications held by Monitoring Technology Ltd, United Medical and Dental School, St Thomas's Hospital, London SE1 7EH.)

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EFFECT OF BOLUS DOSES OF MIDAZOLAM ON INTRACRANIAL PRESSURE AND CEREBRAL PERFUSION PRESSURE IN PATIENTS WITH SEVERE HEAD INJURY

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SUMMARY

We have studied the effects of bolus doses of midazolam 0.15 mg kg^{-1} i.v. on intracranial pressure (ICP), mean arterial pressure (MAP) and cerebral perfusion pressure (CPP) in 12 patients with severe head injury (Glasgow Coma Scale score ≤ 6). The study was performed in patients aged 17-44 yr who were sedated (phenoperidine $20 \mu\text{g kg}^{-1} \text{ h}^{-1}$) and paralysed (vecuronium 2 mg h^{-1}). Midazolam reduced MAP from 89.0 mm Hg to 75.0 mm Hg ($P < 0.0001$), while CPP decreased from 71.0 mm Hg to 55.8 mm Hg ($P < 0.0001$). During the study, CPP decreased to less than 50 mm Hg in four patients. Midazolam induced small, non-significant changes in ICP. However, when control ICP was less than 18 mm Hg ($n = 7$ patients), an increase in ICP was observed. The remaining five patients (control ICP $\geq 18 \text{ mm Hg}$) exhibited a slight decrease in ICP. These findings suggest that bolus administration of midazolam should be performed with great caution in patients with severe head injury, especially when ICP is less than 18 mm Hg . (Br. J. Anaesth. 1993; 71: 267-271)

KEY WORDS

Brain: blood flow, injury, intracranial pressure. Hypnotics, benzodiazepines: midazolam.

The aim of intensive medical treatment in patients with severe head injury is to prevent secondary cerebral damage with the maintenance of an adequate cerebral perfusion pressure (CPP). This may be undertaken by controlling intracranial pressure (ICP) and maintaining adequate mean arterial pressure (MAP), blood-gas tensions, temperature, blood glucose and electrolyte concentrations and serum osmolality. Nociceptive stimulation may induce an increase in ICP, and thereby decrease CPP, which may induce cerebral ischaemia. Muscle paralysis, adequate analgesia and sedation are usually used to prevent such undesirable increases in ICP [1].

The introduction of infusions of hypnotic drugs for the treatment of post-traumatic ICP increases, as a measure to minimize secondary brain damage, has led to a search for agents with a shorter duration of action and fewer side effects than barbiturates. Midazolam is a water-soluble, short-acting benzodiazepine that has been recommended as an effective

i.v. agent for induction of anaesthesia [2]. Its effects on ICP and CPP are not known in patients with severe head injury and have been studied only in neurosurgical patients [3, 4]. However, these findings may not be applicable to head-injured patients, as they were performed on patients with focal intracranial lesions before elective neurosurgical procedures. In many of these patients, ICP was normal and intracranial compliance may have been sufficient to allow some increase in intracranial volume without change in ICP. The aim of this work was to study, in patients with head injury, the effects of an i.v. bolus injection of midazolam on ICP, MAP and the resulting CPP.

PATIENTS AND METHODS

After approval by our local Committee for the Protection of Human Subjects, informed consent was obtained from members of the patients' families.

Patients

The study was performed in 12 patients (11 male) admitted to the Intensive Care Unit (ICU) with severe head injury (Glasgow Coma Scale score ≤ 6). Intracranial pathologies are shown in table I, which also lists age, diagnosis, initial GCS score, associated injuries and outcome of the study patients. The mean weight of the patients was 69.5 (SD 9.6) kg. Throughout the study, the patients were lying in the horizontal position with 15° of head elevation.

Monitoring

A radial artery was cannulated to measure MAP continuously and to allow blood sampling (Merlin 1092-A, Hewlett Packard, Andover, MA). The zero reference for the arterial transducer was the mid-axillary line. Pa_{O_2} and Pa_{CO_2} were measured before and 10 min after injection of midazolam (Ciba Corning B 288, Ciba Corning diagnostics corp., Medfield, MA). Intracranial pressure was measured with a digital pressure monitor (mod. 420, Camino

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TABLE I. Patient data. SAPS = Simplified Acute Physiology Score; ISS = Injury Severity Score

Patient No.	Age (yr)	Initial GCS score	Intracranial pathology	Associated injuries	ISS	SAPS	Stay in ICU (days)	Outcome
1	44	4	Extradural haematoma Cerebral contusion	Fracture of femur	29	15	25	Good recovery
2	28	4	Cerebral contusion		25	7	31	Good recovery
3	28	5	Haemorrhagic cerebral contusion Extradural and subdural haematoma	Liver laceration Fracture of pelvis	50	12	19	Severely disabled
4	30	6	Extradural haematoma Cerebral contusion	Fracture of femur Pulmonary contusion	35	17	13	Moderately disabled
5	32	6	Subdural haematoma Cerebral contusion	Fracture of tibia	34	13	19	Moderately disabled
6	32	6	Extradural haematoma Cerebral contusion	Fracture of femur	35	15	18	Good recovery
7	17	6	Cerebral contusion	Fracture of tibia	34	16	55	Severely disabled
8	29	4	Cerebral contusion Extradural haematoma		25	16	21	Moderately disabled
9	18	6	Cerebral contusion		25	7	14	Good recovery
10	19	3	Cerebral contusion Extradural haematoma	Fracture of femur	34	11	15	Moderately disabled
11	30	6	Cerebral contusion		29	8	13	Good recovery
12	32	6	Cerebral contusion	Fracture of femur	44	13	14	Moderately disabled
Mean	28.3	5.2			33.3	12.5	21.4	
SD		1.1			7.7	3.6	11.9	

Laboratories, San Diego, CA) which utilizes an intracranial bolt and sterile miniature intraparenchymal pressure transducer [5]. The study was performed less than 48 h after insertion of the device. Cerebral perfusion pressure was calculated automatically by the monitor as $CPP = MAP - ICP$ and displayed continuously. Continuous records of MAP and ICP were stored on computer (Vectra, Hewlett-Packard, Andover, MA).

Clinical management

All patients received the same standard management, including immediate neurological assessment by Glasgow Coma Scale score, early diagnosis and treatment of mass lesions, followed by intensive treatment and monitoring. Computerized tomography was performed on admission, 2–4 days later and whenever indicated. All patients received intermittent positive pressure ventilation to maintain Pa_{CO_2} in the range 3.3–4.3 kPa. Inspired oxygen concentration was adjusted to maintain Pa_{O_2} in the range 10.0–20.0 kPa.

Patients were sedated using a continuous i.v. infusion of phenoperidine $20 \mu g kg^{-1} h^{-1}$ and para-

lysed with a continuous infusion of vecuronium $2 mg h^{-1}$. Fluid intake was restricted to 5% glucose $1 ml kg^{-1} h^{-1}$. No colloidal solutions, vasoactive agents, steroids or prophylactic anticonvulsants were administered before or during the study. Measurements were performed in stable patients, at least 12 h after the last infusion of mannitol or frusemide.

The effects of an i.v. bolus dose of midazolam $0.15 mg kg^{-1}$ over a 1-min period on MAP, ICP and CPP were investigated. Midazolam was administered by manual injection into an infusion given continuously via a peripheral i.v. cannula. From the computer recordings, the following measurements were made: MAP, ICP and CPP in the 5 min before injection of midazolam (control values for MAP, ICP and CPP); MAP, ICP and CPP at 1-min intervals for 15 min after administration of midazolam; MAP, ICP and CPP at 5-min intervals for the next 15 min.

Statistical analysis

Statistical calculations were performed using the Statistical Analysis System software package (SAS Institute, Cary, NC) [6]. Two-way analysis of

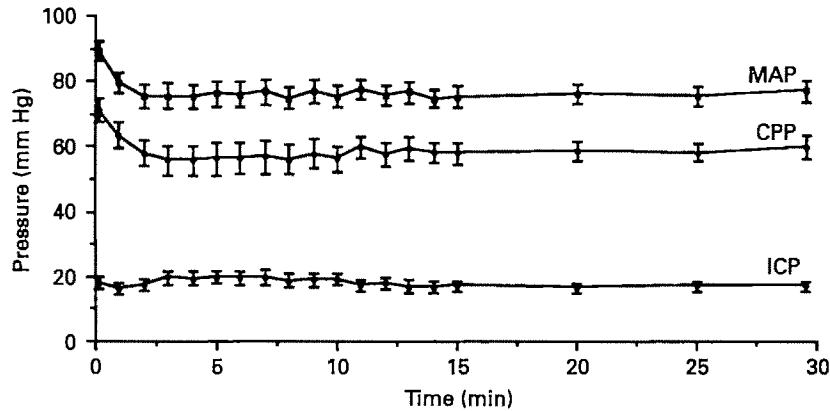


FIG. 1. Mean (SEM) MAP, CPP and ICP before (time 0, control) and after administration of midazolam 0.15 mg kg^{-1} i.v. Changes from control values statistically significant for MAP and CPP.

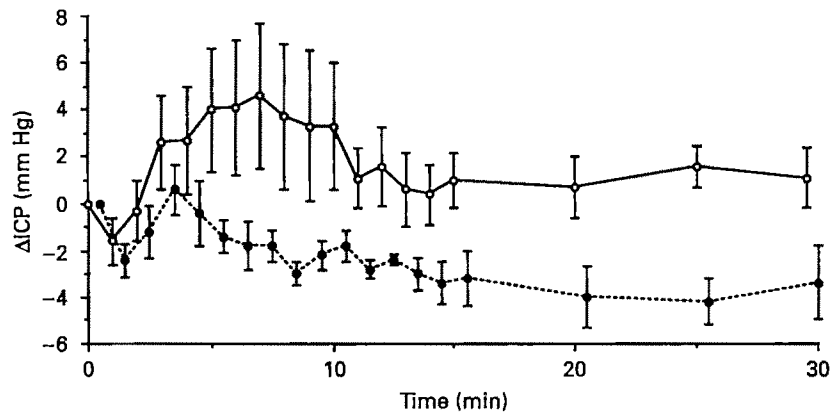


FIG. 2. Mean (SEM) change in ICP before (time 0, control) and after administration of midazolam to patients with control ICP $< 18 \text{ mm Hg}$ (○) and those with control ICP $\geq 18 \text{ mm Hg}$ (●).

TABLE II. P_{aO_2} and P_{aCO_2} values at the time of injection of midazolam 0.15 mg kg^{-1} i.v. (Initial) and 10 min after injection (Final)

Patient No.	Initial P_{aO_2} (kPa)	Final P_{aO_2} (kPa)	Initial P_{aCO_2} (kPa)	Final P_{aCO_2} (kPa)
1	11.6	10.7	4.1	4.0
2	18.9	19.9	4.0	4.1
3	15.9	13.0	3.7	3.2
4	10.0	9.5	4.3	4.4
5	16.1	15.2	3.9	4.0
6	16.1	16.5	3.7	3.7
7	16.8	16.0	3.9	3.7
8	13.9	17.5	4.3	3.6
9	10.0	10.4	4.3	4.4
10	15.9	15.2	3.3	3.7
11	13.7	14.0	4.3	4.4
12	16.9	15.3	3.6	4.0
Mean	14.7	14.5	3.9	3.9
SD	2.8	3.1	0.3	0.4

variance and Dunnett's *t*-test for multiple comparisons were used to determine significant variations with time. Paired variables were compared using Student's *t* test. $P < 0.05$ was considered significant.

RESULTS

Changes in ICP, MAP and CPP are presented in figure 1. The bolus dose of midazolam induced a significant decrease in MAP ($P < 0.0001$). MAP decreased nine of 12 times after midazolam injection;

in six patients this decrease was 10–20% of MAP control and in two other patients the maximal decrease was 20–30%. In the remaining patient, the maximal decrease in MAP reached 50% of control value. Despite these decreases in MAP, ICP increased slightly (not significant). Four of 12 bolus doses of midazolam were followed by a sustained increase in ICP greater than 5 mm Hg.

As a result of these changes in ICP and MAP, CPP was significantly reduced from the second 1 min after injection to the end of the study period ($P < 0.0001$). The decrease in CPP was observed in all patients, and was occasionally severe; it decreased to less than 50 mm Hg for 4 min in two patients and for 28 min in two others. One of the 12 patients had a CPP less than 50 mm Hg before administration of midazolam; CPP remained stable after the injection of midazolam. The magnitude of variation in the different variables (MAP, ICP, CPP) was evaluated according to the control value of ICP. We divided the patients into two groups: group 1 ($n = 7$) with a control ICP less than 18 mm Hg; group 2 ($n = 5$) with an ICP greater than or equal to 18 mm Hg. Although baseline values of MAP were not different between the two groups, analysis of variance showed that in group 1 the decrease in MAP was more pronounced than in group 2 ($P < 0.0001$). CPP was decreased significantly in both groups, with the change more pronounced in group 1 than in group 2.

($P < 0.0001$). This was related probably to the increase in ICP observed in group 1, compared with the slight decrease observed in group 2 ($P < 0.0001$) (fig. 2). Table II shows Pa_{O_2} and Pa_{CO_2} measurements obtained before and 10 min after the injection. There were no significant changes over the period of the study.

DISCUSSION

In managing patients with severe head injury, it is essential to use techniques and pharmacological agents which do not modify ICP and CPP unfavourably. Etomidate has been studied in patients with intracranial lesions [7] and in patients with severe head injury [8, 9]. ICP decreased, but CPP decreased slightly because of the effect of etomidate on MAP. Propofol has also been studied in such patients [10]: ICP was reduced, but CPP decreased because of its effects on MAP. Because of their profound cerebral vasoconstrictive and metabolic depressant effects, barbiturates have been used widely as the drugs of choice for induction of anaesthesia or sedation in patients with intracranial hypertension. The ability of thiopentone to reduce cerebral blood flow (CBF), cerebral metabolic rate and ICP has been demonstrated by Nordström and colleagues [11]. These actions are not entirely dependent of the action of thiopentone on CPP. Shapiro and colleagues [12] reported that ICP has been shown to decrease significantly in patients with intracranial hypertension, but not in patients with normal ICP.

There have been several reports of the effects of benzodiazepines on ICP and CPP. Tateishi and colleagues [13] have shown that diazepam causes a decrease in MAP and CPP. ICP was maintained when baseline values of ICP were greater than 15 mm Hg, but when baseline values were less than 15 mm Hg, ICP decreased. However, this study was conducted in neurosurgical, but non-injured patients. Midazolam may offer some advantages compared with other benzodiazepines because it is a short-acting, water-soluble agent associated with cardiovascular stability [14]. In humans, induction of anaesthesia with midazolam results in a slight decrease in arterial pressure and an increase in heart rate to a degree similar to those after hypnotic doses of thiopentone [15]. Some experimental studies have concluded that midazolam causes a decrease in cerebral oxygen consumption ($CMRO_2$) and CBF [16–19]. In healthy volunteers, Forster and colleagues [20] have shown a decrease in CBF and an increase in cerebrovascular resistance after a bolus injection of midazolam 0.15 mg kg^{-1} , but there are conflicting data: Wolff [21] found that the decrease in CBF was a result of cerebral metabolic depression, rather than a direct vasoconstrictive effect of midazolam. However, in contrast with these experimental studies, midazolam has been found to cause minimal changes in CBF and $CMRO_2$ when administered to patients anaesthetized with nitrous oxide-fentanyl [22]. However, midazolam-induced decreases in CBF and $CMRO_2$ are dose-dependent only to a certain degree, until a plateau effect has been established [19]. It is assumed that the onset of this plateau effect reflects a saturation of the benzo-

diazepine receptors. Fleisher and colleagues did not note an effect of midazolam on ICP in dogs without intracranial hypertension [19].

Few studies have been conducted on the effects of midazolam on ICP and CPP [3, 4, 23]. Moreover, these studies included patients without head injury. After a bolus injection of midazolam 0.2 mg kg^{-1} , Mastronardi and colleagues [3] found a decrease in ICP associated with a slight decrease in MAP in spontaneously breathing patients with various intracranial lesions. Rosa and colleagues [4] did not find significant changes in ICP, MAP or CPP after a bolus injection of midazolam 0.2 mg kg^{-1} in patients with intracranial space-occupying lesions and undergoing artificial ventilation. Giffin and colleagues [23] showed that, in patients undergoing elective surgery and with initial small ICP (12 (sd 1) mm Hg), there was no difference between thiopentone and midazolam in their effects on MAP, CPP and ICP. In our study, we have shown that in head-injured patients, midazolam produced little change in ICP, but a significant decrease in CPP, caused mainly by a decrease in MAP. These effects were more pronounced in patients with ICP $< 18 \text{ mm Hg}$ and could be related to better preservation of the autoregulatory response of the brain. When pressure autoregulation is maintained, a sudden reduction in MAP is likely to induce cerebral vasodilatation and consequently an increase in ICP. Although the critical value of CPP after administration of midazolam has not been determined, CPP was less than 50 mm Hg in five patients in the present study. This is considered the lower limit of autoregulation of CBF in hypertensive patients. It has been reported that a decrease in CPP to this lower limit could be tolerated well in terms of flow-metabolism coupling and intracranial pressure in head trauma patients [24]. However, after severe brain injury, the critical value of CPP could be significantly greater than that of normal patients and a value of 70 mm Hg has been suggested [25]. At values smaller than this threshold, CBF velocity is impaired markedly.

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CEREBROVASCULAR CARBON DIOXIDE REACTIVITY DURING EXPOSURE TO EQUIPOTENT ISOFLURANE AND ISOFLURANE IN NITROUS OXIDE ANAESTHESIA

S. STREBEL, M. KAUFMANN, M. BAGGI AND U. ZENKLUSEN

SUMMARY

We have studied the effects of hypocapnia on cerebrovascular changes in two MAC-equivalent anaesthetic regimens, using the transcranial Doppler technique as an index of cerebral blood flow (CBF) in 24 healthy ASA I patients undergoing spinal surgery. Eight of the patients were subjected to carbon dioxide reactivity challenges in the awake state. Before surgery, the other 16 patients received, in random order, either 1.15% isoflurane in oxygen or 0.5% isoflurane with 70% nitrous oxide. Carbon dioxide reactivity was calculated for each group as the increase in flow velocity per kPa change in PE'_{CO_2} ($cm\ s^{-1}\ kPa^{-1}$). It was significantly greater for the isoflurane group (14.09 (SD 2.44) $cm\ s^{-1}\ kPa^{-1}$) and significantly less for the isoflurane–nitrous oxide group (7.95 (1.32) $cm\ s^{-1}\ kPa^{-1}$) compared with the awake group (11.24 (0.95) $cm\ s^{-1}\ kPa^{-1}$). We conclude that cerebrovascular responsiveness to changes in arterial carbon dioxide concentration is influenced markedly by the anaesthetic procedure. Hyperventilation is more likely to affect CBF during isoflurane anaesthesia than during an MAC-equivalent isoflurane–nitrous oxide anaesthesia. (Br. J. Anaesth. 1993; 71: 272–276)

KEY WORDS

Anaesthetics, volatile: isoflurane. Anaesthetics, gases: nitrous oxide. Brain: blood flow, carbon dioxide tension. Carbon dioxide: hypocapnia. Monitoring: transcranial Doppler.

All volatile anaesthetics have cerebral vasodilator properties that produce a dose-dependent increase in cerebral blood flow (CBF) [1]. Administration may cause potentially dangerous increases in CBF, and hence intracranial pressure, in some neurosurgical patients. However, these changes in CBF may be attenuated effectively by controlled hyperventilation [2]. In addition to hypocapnia, many clinicians use volatile anaesthetics in combination with nitrous oxide, thereby reducing the concentration of volatile drug required.

Isoflurane is considered increasingly for neurosurgical anaesthesia because it has weaker cerebral vasodilator properties than other halogenated anaesthetic agents [3–5]. In neuroanaesthesia, nitrous oxide is used commonly in combination with isoflurane during moderate hypocapnia. Because hyper-

ventilation is an almost universal component of neurosurgical anaesthesia, with or without nitrous oxide, it is essential to understand the relationship between CBF and arterial carbon dioxide concentration during anaesthetic conditions. The present study was designed to compare carbon dioxide responsiveness during two anaesthetic regimens—either isoflurane alone or an MAC-equivalent isoflurane–nitrous oxide combination, with the awake state. The anaesthetic-induced CBF changes were assessed using a transcranial Doppler (TCD) technique.

PATIENTS AND METHODS

We studied 24 ASA I patients (mean age 36 yr (range 24–54 yr); 14 male) undergoing elective spinal surgery. No patient had signs or symptoms of neurological or cerebrovascular disease. Informed consent was obtained and the study was approved by the Committee for Ethics in Human Research of our institution. All patients had fasted for at least 8 h and were unpremedicated. Monitors were used to record ECG (Spacelabs), arterial pressure, non-invasive (Spacelabs) and percutaneous oxygen saturation (Sp_{O_2}) (Nellcor 2500). A carbon dioxide analyser and a multigas analyser (Nellcor 2500) were incorporated in the breathing system to monitor end-tidal carbon dioxide partial pressure (PE'_{CO_2}) and concentrations of nitrous oxide and isoflurane.

Cerebral blood flow velocity was measured for each subject both with and without carbon dioxide challenge; all measurements were made before surgery. Eight of the patients were subjected to carbon dioxide reactivity measurements in the awake state before induction of anaesthesia. The remaining 16 patients were allocated randomly to receive a 1-MAC anaesthetic gas concentration comprising isoflurane alone or isoflurane and nitrous oxide.

Anaesthesia was induced with a single dose of propofol $1\ mg\ kg^{-1}$ and alfentanil $20\ \mu g\ kg^{-1}$; vecuronium $0.1\ mg\ mg^{-1}$ was given for neuromuscular block. The lungs were ventilated for 4 min with isoflurane in oxygen, the trachea intubated and thereafter the lungs ventilated mechanically (Sulla

direct measurement of capillary flow velocity by dynamic capillaroscopy [13], LDF gives an accurate measurement of the microvascular blood flow in septic patients with microcirculatory disturbances. According to Tenland, Salerud and Nilsson [14], the error in this technique is less than 6%. In the present study, we used two different sites for measuring skin capillary blood flow to assess changes in different regions. We believe that change in LDF at the forehead is more important than change in the forearm, because this area is better perfused and more susceptible to alterations in flow than most other areas of the body [15].

Our current state of understanding of the regulation of microcirculation is incomplete. Local, regional and global regulation mechanisms contribute to control of tissue perfusion. The common microcirculatory dysfunctions during CPB are reduced arteriolar flow and endothelial swelling. A recent study has shown that volume loading with hypertonic saline solution in the pre-bypass period prevented deterioration in capillary skin perfusion observed in a non-volume-treated control [16]; this probably resulted from avoidance of endothelial swelling. Poorly perfused capillaries cause liberation and activation of various mediators by which cell adhesion and vasoconstriction are promoted and a vicious cycle of progressive tissue damage is instituted [2]. The hypothesis that the intestinal organs become ischaemic during CPB is supported by a study by Fiddian-Green [17]. Maintenance of the oxygen supply to "vital" organs is accomplished by peripheral resistance, evident most readily in the skin.

There are many causes of acidosis during CPB and the appropriate value of pH during bypass has been controversial for the past 20 years. Nevertheless, maintenance of a "normal" pH is a common objective during CPB. Blood pH tends to decrease during bypass, because of inadequate perfusion and activation of various mediator systems (whole body inflammatory syndrome [18]).

Sodium bicarbonate is known to reduce arterial pressure by peripheral vasodilatation [19] and is said to produce negative inotropic effects [20]. In this study it caused a decrease in arterial pressure for only 5 min, but the microcirculatory blood flow was increased. Thus pressure is not the only important aspect to monitor during CPB; flow-related variables appear to be of equal importance. In addition to macrohaemodynamic influences (arterial pressure, CI), the vascular endothelium appears to be involved in adjustments of tissue perfusion [21–23]. Another aspect of flow regulation—rheological resistance offered by blood and its constituents (haemoglobin concentration, osmolality, viscosity)—remained stable during the present study. Thus it may be assumed that local factors, such as endothelium-dependent vascular relaxation (mediated by endothelium derived relaxant factor (EDRF), nitrous oxide or other factors) might be impaired by acidosis, suggesting abnormalities of local vasomotor regulation. Consequently, correction of acidosis may result in normalization of these regulatory mechanisms as seen by an instant increase in skin capillary

blood flow in the present study. Furthermore, McGillivray-Anderson and Faber [24] demonstrated experimentally that α_2 -adrenoceptor mediated (microvessel) vasoconstriction may be selectively sensitive to reduction in tissue pH (from 7.4 to 7.1). They concluded that this marked sensitivity of α_2 constriction to tissue acidosis may be involved in abnormalities of microcirculatory tissue perfusion.

We cannot deduce from this study if recruitment of capillaries, improved (more homogenous) flow in the capillary network or direct changes in the microvascular tone were responsible for the increase in LDF. According to Gaethgens [23], the degree of heterogeneity may be one important aspect by which the relationship between flow and tissue oxygenation can be modified.

It is not evident from our study if positive effects of NaHCO_3 on the cutaneous microcirculation are predictors of clinical efficacy. Waxman and co-workers [25] showed that absolute LDF values were not predictive for absolute CI values, but LDF clearly reflected acute changes in CI. As shown by the present results, CI is not the only factor that influences microcirculatory blood flow. Although the pump of the extracorporeal circuit maintained a stable CI, LDF changed significantly in the two groups. It has been suggested that monitoring of cutaneous microcirculatory blood flow by the laser Doppler technique may provide useful information on total body perfusion in the critically ill patient [26, 27].

In the present study, the investigation was limited to 15 min after correction of acidosis. After this period, several variables such as additional fluid, rewarming, additional anaesthetics and other factors make interpretation of microcirculatory blood flow assessed by laser Doppler technique very difficult or even impossible. Nevertheless, LDF remained increased after correction of acidosis until the end of CPB.

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REVIEW ARTICLE

EFFECT OF RENAL FAILURE ON DRUG METABOLISM BY THE LIVER

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The selection of a safe and effective drug regimen for patients with renal impairment can be difficult. Data obtained from studies in people with normal renal function cannot be used to design drug therapy for patients with renal failure. Many drugs and their metabolites undergo renal excretion and therefore accumulate in renal failure. However, relatively little is known about the effect of renal impairment on drug metabolism by the liver.

Drug metabolism is a process that alters the pharmacological activity and rate of excretion of a drug. It consists of reactions such as oxidation and reduction in which a new functional group is created, and conjugation reactions in which substances such as glucuronic acid, sulphate or amino acids are added to the molecule. The product of metabolism is generally inactive and water soluble, and can be excreted in either the urine or the bile. Renal failure may influence hepatic drug metabolism either by inducing or inhibiting hepatic enzymes, or by its effects on other variables such as protein binding, hepatic blood flow and accumulation of metabolites. This review examines all of these effects.

In order to investigate the effect of renal impairment on hepatic drug metabolism *in vivo*, it is necessary to study the effect of renal disease on systemic metabolic clearance. A brief outline of clearance and other pharmacokinetic variables examined in this review is given below.

PHARMACOKINETIC CONCEPTS

Clearance

Clearance is the term used to describe the intrinsic ability of the body to clear a drug from the blood or plasma. Total clearance is defined as the volume of plasma which can be cleared of a drug by the metabolizing organ, or the kidney, in unit time. Clearance is additive and total clearance includes renal, metabolic and biliary clearances:

$$Cl_T = Cl_R + Cl_M + Cl_B$$

where Cl_T = total clearance; Cl_R = renal clearance; Cl_M = metabolic clearance; Cl_B = biliary clearance. Metabolic clearance can usually be taken to represent hepatic metabolism, although a few drugs such as

midazolam [73], morphine [17] and propofol [31, 39] undergo substantial extrahepatic metabolism. Biliary clearance is usually small so, for most drugs, if total and renal clearances are known, metabolic clearance can be calculated from the difference. However, there are some drugs such as acebutolol [51] that undergo active biliary secretion and have significant biliary clearances which must be measured.

Total clearance is inversely proportional to the area under the plasma concentration *vs* time curve (AUC). As AUC depends also on the dose of drug administered, total clearance may be defined by the following equation:

$$Cl_T = \frac{\text{Dose}}{\text{AUC}}$$

Systemic metabolic clearance depends on hepatic blood flow, the plasma concentration of unbound drug and intrinsic hepatic clearance. Intrinsic hepatic clearance is the theoretical maximum clearance of unbound drug by the liver in the presence of unrestricted hepatic blood flow [50] and is therefore a measure of the metabolic capacity of liver enzymes. Intrinsic clearance can be changed only by alterations in the metabolizing enzymes (induction or inhibition), so any differences in intrinsic clearance observed in patients with renal failure compared with healthy controls signifies a change in enzyme function.

Metabolic clearance can be defined also by the equation:

$$Cl_M = Q_H \times E$$

where Q_H = hepatic blood flow; E = extraction ratio. The extraction ratio is the fraction of drug removed from the blood during its passage through the liver, and is defined by the following equation:

$$E = \frac{C_a - C_v}{C_a}$$

where C_a = arterial concentration of drug; C_v = venous concentration of the drug. Drugs with a large metabolic clearance ($> 1000 \text{ ml min}^{-1}$), such as pro-

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KEY WORDS

Kidney: failure. Pharmacokinetics. Metabolism.

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pranolol and verapamil, have extraction ratios greater than 0.7 and elimination is perfusion-dependent. Drugs with a small metabolic clearance ($< 300 \text{ ml min}^{-1}$), such as diazepam and theophylline, have extraction ratios less than 0.2 and elimination is perfusion-independent. The clearance of most drugs with a small extraction ratio depends on the extent of protein binding and intrinsic clearance [83].

Distribution

Drugs are distributed from the plasma to the tissues and to proteins in the plasma and in the tissues. The extent to which a drug is distributed to the tissues is measured by the volume of distribution (V). This is not a real volume—it refers to the apparent volume of plasma which the dose of a drug would have to occupy in order to obtain the observed plasma concentration. Thus it merely relates the plasma concentration of a drug to the amount of drug in the body at a given time, it may exceed the plasma volume (5 litre) or even the total body water volume (60 litre).

The extent to which drugs bind to plasma proteins varies from 0 % (lithium) to 99 % (warfarin). Acidic and neutral drugs tend to bind to albumin, while basic drugs bind more commonly to α_1 -acid glycoprotein. Free and protein-bound drug both undergo distribution, although protein binding restricts distribution considerably, as only unbound drug can enter cells. An equilibrium exists between bound and unbound drug, and as the unbound fraction is removed from the plasma by elimination, distribution, or both, bound drug dissociates from its binding sites. About 60 % of total body albumin is extravascular, so tissue protein binding also plays an important part in drug distribution.

Half-life

Plasma elimination half-life is the term most commonly used to express the duration of action of a drug; it is defined as the time taken for the plasma concentration of drug to decrease by 50 %, after the distribution equilibrium has been attained. As the elimination of most drugs takes place according to first-order rate kinetics, the rate of decrease of plasma concentration depends on the concentration of drug present in the plasma at time t and the elimination rate constant (k):

$$\frac{dC}{dt} = k \times C$$

The elimination half-life ($T_{1/2}$) is a reflection of the elimination rate constant and is defined by the equation:

$$T_{1/2} = \frac{0.693}{k}$$

The duration of action of a drug depends also on the volume of distribution, as it is the extent of distribution which determines the actual concentration of drug in the plasma that is presented to the clearing organ. Clearance also affects the duration of action of a drug, as it measures the intrinsic ability of the body to eliminate the drug.

TABLE I. Changes in binding of drugs to plasma proteins in patients with renal failure

Drug	Change in binding
Acidic drugs	
Desmethyldiazepam	Decreased [63]
Phenytoin	Decreased [3, 44, 65, 67, 72]
Benzyl penicillin	Decreased [33]
Salicylate	Decreased [3, 33, 57]
Frusemide	Decreased [4, 68]
Warfarin	Decreased [7, 13]
Basic drugs	
Disopyramide	Increased [43]
Vancomycin	Decreased [75]
Thiopentone	Decreased [3, 40]
Morphine	Decreased [67]

Thus the elimination half-life of a drug is governed by its volume of distribution and clearance:

$$T_{1/2} = 0.693 \times \frac{V}{Cl}$$

Bioavailability

When a drug is given orally, the whole dose may not reach the systemic circulation. This is because the drug may be absorbed incompletely from the gastrointestinal tract, or it may undergo presystemic elimination, either in the liver or, less commonly, in the gut mucosa (first pass effect). Bioavailability (F) is the term used to describe the fraction of an oral dose that reaches the systemic circulation. It is usually obtained from the ratio of the AUC after oral and that after i.v. administration of a drug.

FACTORS AFFECTING DRUG METABOLISM IN RENAL FAILURE

Protein binding

In uraemic patients, the binding of many acidic drugs to albumin is reduced [71] (table I). For drugs that are highly protein-bound and have a small extraction ratio, this may lead to an increase in hepatic clearance [3]. The mechanism for the change in protein binding in renal failure is unknown. As the plasma concentration of albumin correlates poorly with the decrease in protein binding, it has been suggested that a conformational change in the albumin molecule in renal failure may reduce its drug binding capacity [49]. Alternatively, accumulation of substances such as drug metabolites, free fatty acids or organic acids may occur, and these may compete with drugs for binding sites on the albumin molecule [32]. No correlation has been found between the decrease in protein binding and the severity of renal impairment as reflected by conventional biochemical markers of renal function such as serum creatinine [44, 67]. Alterations in protein binding in renal failure are not corrected by haemofiltration [70].

Basic drugs bind mainly to α_1 -acid glycoprotein (AAG) and the effect of renal insufficiency on their binding is controversial (table I). Despite the fact that increased plasma concentrations of AAG are found commonly in patients with chronic renal failure [43], the protein binding of basic drugs is largely unaffected by renal insufficiency [71]. How-

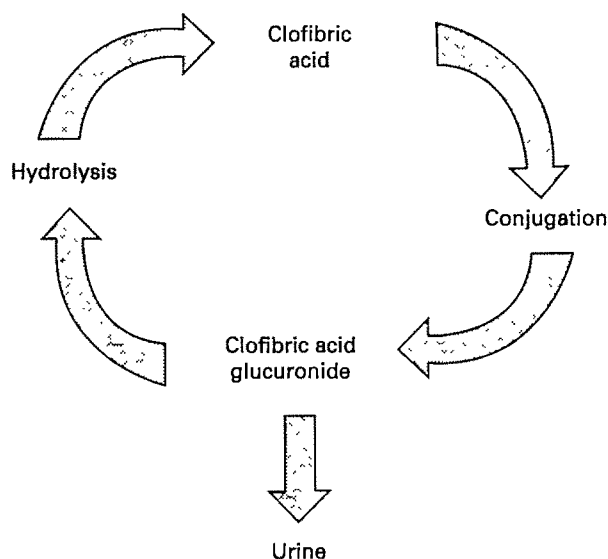


FIG. 1. The "futile" metabolic cycle for clofibrate.

ever, it has been shown that the binding of disopyramide to AAG increases in patients with renal failure [43], and that of vancomycin decreases in patients with end-stage renal failure [75].

Hepatic blood flow

Anaemia secondary to chronic renal failure may lead to a hyperdynamic circulation and an increase in cardiac output and hepatic blood flow [82]. This increases the metabolic clearance of drugs such as morphine and lignocaine that have a large extraction ratio [50].

Oral absorption

Renal failure may affect the intestinal absorption of drugs. For example, the intestinal absorption of frusemide [80] and of pindolol [24] is decreased in patients with impaired renal function. The mechanism for this is unknown, although abnormalities such as increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, inhibition of cell division and a reduction of microvilli in the small intestine have been reported in rats with acute uraemia [59]. This effect must be considered when interpreting the results of pharmacokinetic studies in which drugs are given orally to patients with renal failure.

Accumulation of metabolites

Impaired renal function may also influence drug metabolism through accumulation of metabolites that undergo renal excretion. Drugs with metabolites that accumulate in renal failure, causing a decrease in the rate of elimination of parent drug, include: oxazepam [66], lorazepam [81], paracetamol [60], ketanserin [10] and clofibrate [61]. Accumulated metabolites may compete with the parent drug for active transport into hepatocytes, or they may compete for hepatic enzymes, thus decreasing hepatic clearance of the parent drug [81].

Accumulation of metabolites may lead to an increase in entero-hepatic recirculation, with increased regeneration of the parent drug. For example, when the renal excretion of some glucuronides is impaired, there is a compensatory increase in

TABLE II. Effect of renal failure on the metabolism of drugs and the P-450 isoenzyme responsible for each reaction

Drug	Effect of renal failure on drug metabolism	P-450 isoenzyme
Phenytoin [54, 64]	Increased	2C9, 3A2
Fosinopril [45]	Increased	
Cefpiramide [28]	Increased	
Nifedipine [19]	Increased	3A4
Antipyrine [58, 77]	Increased	1A2B
Suphadimidine [35]	Increased	
Metoclopramide [11, 53]	Decreased	
Nicardipine [1]	Decreased	3A4
Nitrendipine [5]	Decreased	3A4
Verapamil [74]	Decreased	
Nimodipine [48]	Decreased	
Erythromycin [47]	Decreased	3A3/4
Propranolol [15, 56]	Decreased	2D6
Oxprenolol [30]	Decreased	2D6
Bufuralol [8]	Decreased	2D6
Metoprolol [46]	Unchanged	2D6
Lignocaine [26, 79]	Unchanged	3A4
Codeine [41]	Unchanged	2D6
Theophylline [12]	Unchanged	1A2
Nortriptyline [29]	Unchanged	2D6
Isradipine [23]	Unchanged	3A4
Nisoldipine [42]	Unchanged	3A4
Propafenone [21]	Unchanged	2D6

biliary excretion, with regeneration of the parent compound by hydrolysis in the gut. The resulting increase in entero-hepatic recirculation may reduce the net plasma clearance of the parent drug, as seen with oxazepam [66] and paracetamol [60].

The accumulation of metabolites may also lead to regeneration of the parent drug when an oxidation-reduction equilibrium exists between the parent drug and its metabolite [81]. This leads to an increased plasma concentration of the parent drug, which may be confused with a decrease in hepatic clearance. For example, the terminal elimination half-life of ketanserin depends on the rate of elimination of its major metabolite ketanserin-ol, because of a metabolic oxidation-reduction equilibrium between the two compounds. Consequently, accumulation of ketanserin-ol in renal failure leads to regeneration of ketanserin [10]. A similar mechanism is responsible for the "futile" metabolic cycle (fig. 1) seen in analogues of 2-aryl propionic acid compounds such as clofibrate [61] and ciprofibrate [34]. In this cycle, accumulation of the labile acyl glucuronide in renal impairment is followed by deconjugation back to the parent compound and an increase in the plasma concentration of parent compound.

Accumulation of metabolites in uraemic patients may also result in product inhibition, which is thought to contribute to the prolonged elimination half-life of lorazepam in patients with renal failure [81].

Extrahepatic drug metabolism

Renal impairment may influence drug metabolism by an effect on extrahepatic metabolism. The kidneys themselves have an important role in drug metabolism, and many of the drug-metabolizing enzymes found in the liver are present also in the renal cortex

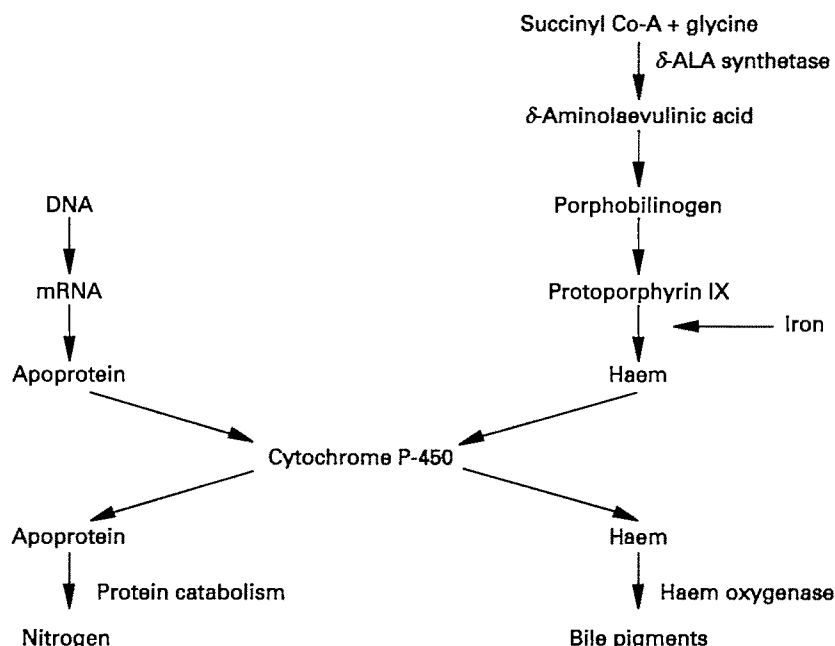


FIG. 2. Synthesis and degradation of cytochrome P-450.

[2]. In a study of patients with unilateral hydronephrosis, it has been shown that there is a reduction in cytochrome P-450 content and in biphenyl-4-hydroxylase activity in the hydronephrotic cortex compared with the normal side [86]. It is thought that drug metabolism is likely to be reduced in other renal diseases characterized by cortical destruction [37]. It is possible also that renal diseases may modify extrahepatic metabolism at other sites such as intestinal mucosa, lungs, brain and skin.

Inhibition of organic acid transport

The transport of organic acids such as hippurate [25] and indocyanine green [84] into the liver has been shown to decrease in renal failure. It is possible, therefore, that the transport of other organic acids necessary for the metabolism of drugs may also be inhibited in patients with impaired renal function. For example, isocitrate and α -ketoglutarate are essential for the regeneration of NADPH, which is needed for substrate hydroxylation by cytochrome P-450, and free fatty acids are necessary for some conjugation reactions. Inhibition of the transport of these organic acids into the liver in uraemic patients may lead to a decrease in hepatic clearance.

Anion depletion

Long-term or high-dose paracetamol therapy has been shown to be associated with a decreased rate of sulphate conjugation in patients with renal dysfunction [60]. This is thought to be caused by sulphate depletion, despite the fact that chronic renal failure is usually associated with sulphate retention rather than depletion.

Changes in hepatic cytochrome P-450

The results of studies on the effects of renal impairment on hepatic drug metabolism are conflicting. Metabolism has been shown to be increased, decreased or unaffected by renal failure, depending

TABLE III. Possible mechanisms for induction of cytochrome P-450. (Adapted from [36], with permission)

1.	Enhancement of DNA-dependent RNA polymerase.
2.	Increased nucleocytoplasmic transport of ribonucleoprotein.
3.	Increased synthesis or stability of mRNA coding for cytochrome P-450.
4.	Increased haem biosynthesis.
5.	Decreased apoprotein degradation.
6.	Decreased haem degradation.

on the drug and animal species studied. One problem with these studies is the possible interaction of other variables that could alter the rate of hepatic drug metabolism. Of particular importance are concurrent drug administration, age, smoking habit and alcohol intake. Furthermore, the possibility of pharmacogenetic variation must be considered when interpreting the results of these studies.

Induction. Several studies have shown that the hepatic clearance of certain drugs is increased in renal failure (table II). However, the exact mechanisms for this have yet to be elucidated. An increase in hepatic clearance is generally indicative of increased hepatic drug metabolism, provided that changes in other pharmacokinetic parameters, such as protein binding and bioavailability, have been excluded. If substrate concentration is constant, an increase in hepatic metabolism results from induction of the hepatic drug-metabolizing enzymes, notably the cytochrome P-450 system. Enzyme induction may be caused by increased synthesis of cytochrome P-450 enzymes, decreased degradation or activation of pre-existing components of the cytochrome P-450 system [36]. Figure 2 shows the pathways of cytochrome P-450 synthesis and catabolism. The possible molecular mechanisms of induction are listed in table III. The cytochrome P-450 system consists of a number of isoenzymes that have been classified into families and subfamilies.

Renal failure may affect isoenzymes differently, which may explain why the effect of renal disease on drug metabolism varies for different drugs.

It has been suggested that, in renal failure, a substance(s) that induces certain cytochrome P-450 isoenzymes builds up in the plasma. Initially, it was thought that an enzyme inducer might be leached out of dialysis tubing [38]. However, this was not supported by the finding that accelerated antipyrine metabolism occurred in all patients with chronic renal failure, irrespective of whether they had received haemodialysis [58]. Another theory is that the enzyme inducer present in uraemic plasma is a dietary substance, or a metabolite of a dietary substance, that accumulates in renal failure [69]. For example, cruciferous plants contain naturally occurring indoles, that are potent inducers of microsomal hydroxylation in the rat [55].

Two studies [54, 64] have shown that, after phenytoin is given i.v., the elimination half-life is significantly shorter in patients with renal failure than in patients with normal renal function. This could not be explained by a change in volume of distribution, as decreased protein binding resulted in an increase in volume of distribution in both studies. It was suggested, therefore, that the decrease in elimination half-life was caused by induction of phenytoin metabolism, leading to an increase in metabolic clearance.

Fosinopril is a new angiotensin-converting enzyme inhibitor that undergoes dual elimination through the liver and the kidney. It has been shown that, as renal function declines, there is a compensatory increase in the hepatic clearance of fosinopril when it is given i.v. [45]. Thus, although renal clearance decreases, total clearance remains unchanged. Accelerated metabolism and increased biliary excretion were shown to account for the increased hepatic clearance in this study. Similarly, although the renal clearance of i.v. cefpiramide has been shown to decrease linearly with creatinine clearance, total clearance remained unchanged because of a compensatory increase in metabolic clearance [28].

Nifedipine, which is eliminated almost entirely by metabolism, has a significantly greater total clearance in patients with terminal renal failure after oral and i.v. administration [19]. No differences in protein binding were found between healthy volunteers and patients with renal failure, and the increased total clearance may represent an increase in metabolism. However, all the patients in this study were receiving long-term haemodialysis and it has been suggested that this may increase splanchnic blood flow [9]. As nifedipine has a large extraction ratio, an increase in hepatic blood flow could account for the increased hepatic clearance seen in this study.

Another drug oxidized by the liver that has accelerated metabolism in patients with renal failure is antipyrine. It has been shown that the plasma half-life of antipyrine given i.v. is decreased in patients with chronic renal failure [58]. As there was no significant difference between the volume of distribution in patients with chronic renal failure and normal subjects, the decrease in plasma half-life is

likely to reflect an increase in antipyrine oxidation, resulting in an increase in metabolic clearance. A possible mechanism suggested for this is enzyme induction by endogenous or dietary substances that accumulate in renal failure. Accumulation of substances found in the water supply, such as insecticides, may also cause enzyme induction [27]. This could be especially important in patients receiving haemodialysis, who are exposed to large volumes of water. Conflicting results have shown that, when antipyrine is given orally to patients with renal failure, demethylation of antipyrine is decreased, while hydroxylation is unchanged [77]. This shows that renal failure affects not only individual drugs differently, but also each metabolic pathway by which they are eliminated.

Conjugation reactions have been studied in rats that have undergone bilateral nephrectomy [22]. This work showed that glucuronyl transferase activity is increased in acute uraemia, and suggests that the mechanism may be induction by phenols that accumulate in renal failure. Phenols and other uraemic toxins are inactivated by glucuronidation, so this may be an important homeostatic mechanism. Increased hepatic acetylation of sulphadimidine, another conjugation reaction, occurs in patients with chronic uraemia [35], although this effect is reversed by long-term haemodialysis. The mechanism for this may be induction of hepatic acetylation by amines that accumulate in uraemia and are removed by haemodialysis. Acetylation is an important detoxification pathway for many amines, so the increase in acetylation in patients with renal failure may be a compensatory response.

Inhibition. In contrast to the above findings, the metabolic clearances of other drugs appear to be decreased in patients with impaired renal function (table II); this may result from inhibition of hepatic drug-metabolizing enzymes. Inhibition of cytochrome P-450 isoenzymes may be caused by inhibition of enzyme synthesis, increased destruction of pre-existing enzymes, formation of drug-enzyme complexes that inactivate the enzyme, or depletion of cofactors. Alternatively, substances may accumulate in renal failure that are competitive inhibitors of drug-metabolizing enzymes. It is not yet known which of these mechanisms is responsible for the inhibition of drug metabolism in renal failure.

It has been suggested that a circulating enzyme inhibitor is present in uraemic plasma in the rat [76]. In that study, livers from healthy rats and rats with uranyl nitrate-induced renal failure, were perfused with either uraemic or non-uraemic blood. In both groups of liver perfused with uraemic blood, the hepatic extraction of propranolol was significantly decreased. The exact nature of the proposed circulating enzyme inhibitor is unknown, although many endogenous substances, such as amines, organic acids, indolic derivatives, amino acids and peptides, accumulate in renal failure and could be responsible for the enzyme inhibition [14, 76].

Another study in rats [52], has shown that the decreased hepatic drug metabolism seen in renal failure may be caused by a deficiency of δ -aminolaevulinic acid, which is necessary for cyto-

chrome P-450 enzymes synthesis. In this study, the decreased cytochrome P-450 content and activity seen in liver microsomes from uraemic rats, could be corrected by treatment of the rats with δ -aminolaevulinic acid.

Two studies have shown that the non-renal clearance of metoclopramide, given i.v., is decreased in patients with renal impairment [11, 53]. The authors suggested that this could be caused by an alteration in entero-hepatic circulation or a decrease in renal metabolism; however, inhibition of hepatic metabolizing enzymes by uraemic plasma could not be excluded.

The plasma clearance of nifedipine, given i.v., is significantly reduced in patients with renal impairment, but restored towards normal in patients with end-stage renal failure undergoing haemodialysis [1]. Less than 1% of a dose of nifedipine is excreted unchanged in the urine, so the decrease in plasma clearance reflects a decrease in metabolic clearance. No significant differences in the measured indices of hepatic function were found to explain the decrease in metabolic clearance in the patients with renal impairment, and accumulation of a dialysable inhibitor of metabolism was suggested as a possible mechanism. Unfortunately, plasma concentrations of nifedipine metabolites were not measured in this study, so product inhibition by accumulation of metabolites cannot be excluded as a cause for the decrease in hepatic clearance.

Studies with nitrendipine, another calcium channel blocker, have shown conflicting results in patients with renal failure. One study [5] found that after 7 days of oral treatment with nitrendipine, the total clearance was less in patients with renal failure than in control patients. As nitrendipine is eliminated almost entirely by hepatic metabolism, it was suggested that the decrease in total clearance reflected a reduction in hepatic extraction. However, intestinal absorption was not measured in this study, so the results must be interpreted with caution. Two similar studies [6, 20] failed to show any changes in the pharmacokinetic parameters of nitrendipine given orally or i.v. to patients with renal failure.

The pharmacokinetics of a single bolus dose of verapamil given orally or i.v. were studied in normal volunteers and patients with advanced renal failure [74]. The mean non-renal clearance was decreased in patients with renal failure whether the drug was given i.v. or orally. However, two other studies [62, 85] did not show any significant changes in the pharmacokinetics of verapamil when it was given orally or i.v. to patients with diminished renal function. Patients in these two studies were receiving regular haemodialysis, whereas it was not stated if this was the case in the first study. Such a difference could explain the discrepancy in the findings.

The AUC of nimodipine given orally increased in patients with impaired renal function [48]. In this study, intestinal absorption was not affected by renal failure, so the increase in AUC may represent a decrease in clearance, which for nimodipine comprises mainly metabolic clearance. However, the patients with renal insufficiency were significantly older than the volunteers, and the difference in age

rather than renal function may have been responsible for the pharmacokinetic changes. Furthermore, a control group was not included in this study; instead, the findings in patients with renal impairment were compared with pre-existing data on the pharmacokinetics of nimodipine in volunteers.

When erythromycin was given orally, the maximum serum concentrations and AUC were found to be greater in patients with renal failure than in healthy control subjects [47]. There was no difference in elimination half-life between the two groups. As the volume of distribution of erythromycin is known to increase in renal failure, the increased plasma concentrations were thought to represent an increase in bioavailability. This could have been caused by either an increase in the extent of intestinal absorption, or a decrease in first pass effect. The former is unlikely, as the time taken to reach the maximum plasma concentration (t_{Cmax}) was the same in both groups. Thus decreased first pass metabolism would seem to be the most likely explanation for the pharmacokinetic changes. This may have been caused by a decrease in hepatic metabolism; however, erythromycin is also metabolized in the gut wall, so a decrease in intestinal metabolism cannot be excluded. The increased bioavailability shown in this study is important clinically, as increased plasma concentrations of erythromycin are associated with ototoxicity. The dose of erythromycin should therefore be decreased in uraemic patients.

The bioavailability of oral propranolol, a drug which undergoes significant first pass metabolism, is greater in patients with chronic renal failure not receiving haemodialysis than in patients receiving haemodialysis or in healthy volunteers [15]. No differences in volume of distribution, elimination half-life, protein binding or absorption were seen between the patient groups, so the increase in bioavailability may be assumed to reflect a decrease in hepatic clearance. Propranolol was not extracted by dialysis, but the difference in bioavailability between the dialysed and non-dialysed groups may be explained by the presence of a dialysable factor in uraemic plasma that inhibits hepatic metabolism. However, another study has shown that the bioavailability of oral propranolol is increased in uraemic patients receiving regular haemodialysis [56]. This study showed that the intestinal absorption of propranolol was not altered by renal insufficiency, and concluded that the increase in bioavailability was caused by a decrease in hepatic metabolism. In another study [78] on the pharmacokinetics of ^{14}C -labelled propranolol, given both orally and i.v., no delay in the conversion of propranolol to its 4-hydroxy or conjugate metabolites was seen in patients with renal failure (not receiving haemodialysis) compared with control subjects. The creatinine clearances of the patients in this study were greater ($2-62 \text{ ml min}^{-1}$) than those in the two previous studies ($0-10 \text{ ml min}^{-1}$), and this may explain the different findings in this study. Studies on the pharmacokinetics of oxprenolol [30] and bufuralol [8] given orally to patients with renal failure, have shown that presystemic elimination is

decreased in uraemia. In both studies, elimination half-life was unchanged, and this was attributed to a concomitant decrease in volume of distribution.

No changes in cytochrome P-450. The hepatic metabolism of some drugs appears to be unaffected by renal impairment (table II). For example, the total clearance of oral and i.v. metoprolol in patients with varying degrees of renal impairment is the same as that in healthy subjects [46]. Metoprolol is eliminated primarily by hepatic biotransformation, and less than 5% of a dose given orally is excreted unchanged by the kidneys. As the renal clearance is so small, it can be assumed that the hepatic clearance of metoprolol was unaffected by renal failure.

Two studies [26, 79] have shown that the total clearance of i.v. lignocaine is unaffected by renal dysfunction. In both these studies, the uraemic patients were receiving chronic haemodialysis. It is possible that haemodialysis may correct abnormalities of drug metabolism, by removing specific plasma inducers or inhibitors of cytochrome P-450 enzymes. It would be useful, therefore, to know the effect of renal failure on the disposition of lignocaine in patients not receiving haemodialysis.

Work on patients receiving regular haemodialysis has shown that the metabolism of codeine, given orally or i.v., is unaffected by renal failure [41]. The metabolism of theophylline, given by continuous i.v. infusion, has also been shown to be normal in patients with renal failure, maintained on haemodialysis [12]. In this study all the patients had chronic airways disease and were smokers, both of which are known to affect hepatic drug metabolism. Although these factors were present in the control group, there were so many variables that interpretation of the results is difficult.

Nortriptyline is metabolized extensively by hepatic demethylation and hydroxylation, followed by conjugation. In patients with renal failure, total clearance after a single oral dose was similar to that in a group of healthy controls, and no correlation was seen between total clearance and glomerular filtration rate [29]. This suggests that the metabolism of nortriptyline is unaffected by renal failure, although the effects of renal failure on protein binding were not assessed. This study included both patients receiving haemodialysis and those who were not.

Isradipine is a new calcium channel blocker that undergoes extensive hepatic metabolism. No differences in clearance were seen when isradipine was given orally to patients with and without renal failure, suggesting that hepatic biotransformation is unaffected by renal dysfunction [23]. However, there was considerable intersubject variability in this study, so the results must be interpreted with caution.

Nisoldipine is another second generation dihydropyridine calcium channel blocker that is eliminated mainly by hepatic metabolism. The oral clearance of nisoldipine in patients with impaired renal function, not receiving haemodialysis, is the same as that in control subjects, indicating that metabolic clearance is unchanged by renal impairment [42].

Work on propafenone [21], a new class I antiarrhythmic agent eliminated primarily by hepatic

metabolism, has shown that its metabolism is unaffected by renal failure.

CONCLUSIONS

Hepatic drug metabolism may be increased, decreased or remain unchanged in patients with renal failure. The exact mechanisms of these changes are unknown, and the effect of renal impairment on the metabolism of a particular drug cannot be predicted from theoretical considerations. For example, nifedipine, nitrendipine and nisoldipine are all apparently metabolized *in vivo* by the same isoenzyme, cytochrome P-450 3A4, yet renal failure increases the metabolism of nifedipine [19], decreases that of nitrendipine [6] and has no effect on the metabolism of nisoldipine [42]. In the light of the present state of knowledge, it is important to consider the possibility of altered hepatic clearance when prescribing drugs for patients with renal impairment. If the metabolism of a drug is known to increase in renal failure, then the dose may have to be increased in order to achieve the therapeutic effect. Conversely, if the metabolism of a drug decreases in renal failure, then a reduction in dose will be necessary to avoid unwanted effects. If the effect of renal failure on the metabolism of a drug is unknown, then particular care should be taken with drugs that are extensively metabolized in the liver and have a narrow therapeutic index. Unfortunately, measuring the plasma concentration of a drug is only helpful for a small number of drugs such as anticonvulsants, antiarrhythmics and certain antibiotics. This is because, for many drugs, the plasma concentration does not correlate with the concentration of drug at the receptor [16] or the therapeutic effect [18]. The dose of any drug that undergoes significant hepatic metabolism should therefore be titrated carefully against the clinical effect when given to a patient with impaired renal function.

Future studies of drug metabolism in renal failure should exclude, as causes of altered hepatic clearance, changes in other pharmacokinetic variables, for example, alterations in protein binding of drugs which are highly protein-bound and changes in hepatic blood flow of drugs with a high extraction ratio. The plasma concentration of metabolites should always be measured to eliminate the effects of metabolite accumulation on hepatic clearance, and the possibility of extrahepatic metabolism should be considered. Future studies should also ensure that the control and study groups are well matched for age, smoking habit and alcohol intake, so that the effects of these factors on hepatic clearance is minimized.

Further research is needed to elucidate fully the mechanisms responsible for the changes in hepatic drug metabolism, observed in renal failure. The possibility that different mechanisms operate for different cytochrome P-450 isoenzymes should be investigated.

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SHORT COMMUNICATIONS

EFFECTS OF DIFFERENT DOSES OF THIOPENTONE ON THE INCREASE IN SERUM MYOGLOBIN INDUCED BY SUXAMETHONIUM IN CHILDREN†

I. NOGUCHI, G. SUZUKI AND Y. AMEMIYA

SUMMARY

We have studied the effects of different doses of thiopentone on the increase in serum myoglobin after administration of suxamethonium during inhalation induction of anaesthesia in children. Forty-three children were anaesthetized with halothane and nitrous oxide in oxygen and allocated to four groups: group S received suxamethonium 1 mg kg^{-1} to facilitate intubation; group ST2 received thiopentone 2 mg kg^{-1} and group ST4 received thiopentone 4 mg kg^{-1} , before administration of suxamethonium 1 mg kg^{-1} ; group N did not receive thiopentone or suxamethonium. Serum myoglobin and creatine kinase (CK) concentrations were measured until 60 min after the injection of suxamethonium. Both myoglobin and CK concentrations increased in the three groups receiving suxamethonium. There were no significant differences between groups S and ST2, but the myoglobin concentration was less in group ST4 than in groups S and ST2. A significant difference in CK concentration was found only between groups ST2 and ST4 at 60 min. In group N, both values remained reasonably constant. Thiopentone 4 mg kg^{-1} , but not 2 mg kg^{-1} , attenuated the increase. The results indicate that to prevent a marked elevation in serum myoglobin after administration of suxamethonium, thiopentone 4 mg kg^{-1} should be administered. (Br. J. Anaesth. 1993; 71: 291–293)

KEY WORDS

Anaesthetics, intravenous: thiopentone. Complications: myoglobin.

I.v administration of suxamethonium to children anaesthetized with halothane is associated with increases in serum concentrations of myoglobin [1–4] and creatine kinase (CK) [2, 3]. Although the inhibitory effects of thiobarbiturates on the release of myoglobin and CK have been reported previously [5], the effects of different doses of thiopentone administered during inhalation induction of anaesthesia have not been described clearly.

We have studied the effects of different doses of thiopentone on changes in serum myoglobin and CK until 60 min after administration of suxamethonium to children anaesthetized with halothane and nitrous oxide.

METHODS AND RESULTS

We studied 43 children of both sexes, aged from 2 yr 11 months to 13 yr 10 months, ASA I, undergoing minor elective urological, orthopaedic or oral surgery under general anaesthesia. All parents had been informed of the nature of the study and informed consent was obtained.

All children received diazepam $0.5\text{--}0.7 \text{ mg kg}^{-1}$ (maximum dose 10 mg) and atropine 0.02 mg kg^{-1} (maximum dose 1.0 mg) orally 1–2 h before induction of anaesthesia. Inhalation induction was performed with halothane and nitrous oxide in oxygen via a face mask. After loss of consciousness, the children were allocated randomly to four groups. The first group received suxamethonium 1 mg kg^{-1} to facilitate tracheal intubation (group S; $n = 12$). The second group (ST2; $n = 10$) received thiopentone 2 mg kg^{-1} and the third group (ST4; $n = 10$) received thiopentone 4 mg kg^{-1} , before administration of suxamethonium 1 mg kg^{-1} . In the fourth group, tracheal intubation was performed without either thiopentone or suxamethonium (group N; $n = 11$). Anaesthesia was maintained with halothane and nitrous oxide in oxygen via a Jackson Rees system. Lactated Ringer's solution was infused at $10\text{--}15 \text{ ml kg}^{-1} \text{ h}^{-1}$ and blood samples were obtained before and at 5, 20 and 60 min after injection of suxamethonium in groups S, ST2 and ST4, and at the same times in group N. The values obtained before the injection of suxamethonium were taken as baseline.

Blood samples were allowed to stand and coagulate for 20–30 min. After centrifugation, all specimens were kept frozen at -20°C until required for assay. Myoglobin concentrations were determined in non-haemolysed sera using a radioimmunoassay (Daiichi Radioisotope, Tokyo, Japan). The range of detection was myoglobin $0.3\text{--}200 \text{ ng ml}^{-1}$. When the value was $> 200 \text{ ng ml}^{-1}$, the serum was diluted 2-, 5- or 10-

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TABLE I. Changes in myoglobin and creatine kinase (CK) concentrations in the four groups (mean (SD), median [range]). Significant differences: vs baseline * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; vs group N † $P < 0.05$, ‡ $P < 0.01$, §§ $P < 0.001$; vs group ST2 † $P < 0.05$, ‡ $P < 0.01$, §§ $P < 0.001$; vs group S † $P < 0.05$, ‡ $P < 0.01$, §§ $P < 0.001$

Group	Myoglobin concentration (ng ml ⁻¹)				CK concentration (iu litre ⁻¹)			
	Baseline	5 min	20 min	60 min	Baseline	5 min	20 min	60 min
S	19 (2)	1346 (559)***§§§	1814 (708)***	2192 (639)***§§§	57.8 (4.1)	67.2 (5.6)*	99.8 (17.5)***	174.0 (32.8)***§§§
	20	677	996	1301	59.6	62.6	70.8	146.1
	[10-38]	[48-7157]	[61-9194]	[117-8088]	[35.9-80.0]	[41.5-107.8]	[53.9-248.2]	[58.5-487.8]
ST2	42 (12)	1011 (256)§§§	1650 (415)§§§	1932 (478)***§§§	72.5 (7.1)	89.6 (11.6)	128.6 (24.0)	251.2 (57.7)*§§
	26	630	920	1100	67.5	76.3	85.6	137.6
	[20-38]	[280-2400]	[410-3600]	[530-4300]	[36.8-89.3]	[44.8-141.4]	[41.8-249.2]	[58.5-483.6]
ST4	19 (2)	520 (136)§§	796 (233)**§§	685 (140)***††	60.7 (4.0)	63.0 (4.5)	75.5 (7.0)	114.0 (15.7)*†
	17	410	540	610	60.6	62.3	79.7	117.0
	[10-35]	[83-1300]	[118-2400]	[165-1400]	[44.8-89.6]	[41.5-89.6]	[39.8-98.0]	[52.3-205.8]
N	22 (8)	25 (3)	27 (3)	40 (12)	72.7 (4.1)	70.1 (4.8)	67.2 (4.3)	62.8 (4.5)
	20	22	27	34	69.1	67.3	67.3	60.7
	[14-43]	[16-43]	[14-46]	[17-153]	[56.4-93.5]	[51.3-89.1]	[48.2-87.3]	[46.3-84.5]

fold. CK concentrations were measured by the modified Rosalki method (Yatoron, Tokyo, Japan).

Statistical analyses were performed using Kruskal-Wallis and Wilcoxon *U* test for myoglobin and CK concentrations, and chi-square and Student's *t* test as appropriate. The results were considered significant when $P < 0.05$. All data are presented as mean (SD).

The four groups were comparable in age, weight and operations performed. The concentrations of halothane administered during induction and maintenance were also comparable.

Changes in myoglobin and CK are shown in table I. Baseline concentrations were normal in all four groups and increased in the three groups receiving suxamethonium. Among those three groups, there were no significant differences between groups S and ST2, the myoglobin concentration was less in group ST4 than in groups S and ST2, and a significant difference in CK concentration occurred only between groups ST2 and ST4 at 60 min.

COMMENT

In 1971 Ryan, Kagen and Hyman reported a greater incidence of myoglobinaemia in children than in adults, after suxamethonium. However, the study used various induction techniques. Tammisto, Leikonen and Airaksinen [5] found that the increases in myoglobin and CK concentrations were less in those receiving barbiturates, in both children and adults.

Reduced increases in serum myoglobin and CK concentrations by barbiturates have been reported in adults [6]: thiamylal 1.5–2.0 mg kg⁻¹ administered after induction of anaesthesia with halothane reduced the increase in myoglobin and CK concentrations to 10–35% of control. In contrast, a dose of thiopentone 2 mg kg⁻¹ was found to be ineffective in the present study. The reasons for this difference could be attributed to age differences in the responsiveness to barbiturates. In addition, the timing of administration, or pharmacological differences between thiopentone and thiamylal should be considered.

In contrast, Sekino and colleagues [7] administered thiopentone 5 mg kg⁻¹ for i.v. induction of anaesthesia in children, and found that the concentration of myoglobin was reduced to about 22% of that without thiopentone. However, they concluded that the dose was ineffective, as a statistically significant difference was not found.

Manani and colleagues [8] reported that thiopentone reduced the incidence of myalgia and fasciculation induced by suxamethonium. It has been reported previously that a correlation does not necessarily exist between the intensity of muscle fasciculation and the degree of muscle damage as indicated by the serum concentrations of myoglobin and CK [2, 3]. This suggests that the increases are not necessarily attributable to muscle cell damage by fasciculation, but rather to accelerated permeability of the muscle cell by depolarization. The mechanism that reduces the rate of increase by barbiturates has not been delineated clearly and requires further investigation. It is possible that the mechanism might involve a cell membrane stabilizing effect,

although an effect on neuromuscular transmission also may play a role.

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I.V. CLONIDINE FOR POST-EXTRADURAL SHIVERING IN PARTURIENTS: A PRELIMINARY STUDY

G. CAPOGNA AND D. CELLENO

SUMMARY

We have studied the efficacy of i.v. clonidine to suppress post-extradural shivering in parturients. Forty healthy parturients who received extradural block for labour (n = 20) or for Caesarean section (n = 20) and who required treatment for shivering after delivery were allocated randomly to two groups. Group I received i.v. clonidine 30 µg diluted in saline to a total volume of 5 ml (therapeutic solution). This bolus was repeated every 5 min if the initial therapy produced no improvement, up to a maximum dose of 90 µg. Group II received saline 5 ml (placebo solution), repeated every 5 min if the initial bolus produced no improvement, up to a maximum of three boluses. After 15 min of observation, patients in group I received the placebo solution and those in group II received the study solution. All patients who received clonidine improved, and 75% ceased to shiver within 5 min after only one dose of clonidine 30 µg. In contrast, none of the patients treated with saline improved. When patients in the placebo group received clonidine, improvement occurred. Arterial pressure, heart rate, core and peripheral temperature and oxygen saturation did not differ significantly between and within the groups before and after administration of clonidine. We conclude that a small dose of i.v. clonidine may be useful to suppress post-extradural shivering in parturients. (Br. J. Anaesth. 1993; 71: 294-295)

KEY WORDS

Anaesthesia: obstetric. Anaesthetic techniques: extradural. Complications: shivering. Pharmacology: clonidine.

The incidence of post-extradural shivering varies widely, but is a well recognized problem. Many treatments have been suggested for this condition and it has been shown that i.v. clonidine effectively suppresses postoperative shivering after general [1] and extradural [2] anaesthesia in non-pregnant patients.

This randomized, double-blind, cross-over study was undertaken to investigate if i.v. clonidine suppresses post-extradural shivering in parturients.

METHODS AND RESULTS

After Ethics Committee approval, informed consent was obtained from healthy parturients who received extradural block for labour or Caesarean section.

Only patients who required therapy for post-extradural shivering after delivery were included in the study. All the patients had shivering accompanied by subjective sensations of cold and discomfort.

Patients who delivered spontaneously (n = 20) received extradural analgesia with 0.125% bupivacaine and 1:800 000 adrenaline. Those who delivered by Caesarean section (n = 20) received extradural anaesthesia with an alkalized solution of 2% lignocaine with 1:200 000 adrenaline. In all patients, extradural block was well established and hypotension, if it occurred, was controlled with an i.v. infusion of ephedrine.

Local anaesthetic solutions and i.v. fluids were stored and administered at room temperature; the ambient temperature was 20.3 °C.

Patients were allocated randomly to two treatment groups. Group I received i.v. clonidine 30 µg diluted in saline to a total volume of 5 ml (therapeutic solution). This bolus was repeated every 5 min if the initial therapy produced no improvement, up to a maximum dose of 90 µg. Group II received saline 5 ml (placebo solution), repeated every 5 min if the initial bolus produced no improvement, up to a maximum of three boluses. After 15 min of observation, patients in group I received the placebo solution and patients in group II received the study solution.

Only complete inhibition of shivering was considered to be an adequate response to the therapy.

Both the patients and the anaesthetist who gave the i.v. solutions and evaluated the patient's response were unaware of the contents of the syringe.

Heart rate (HR) and pulse oximetry were displayed continuously and arterial pressure (AP) was recorded every 2 min using an automatic device (Cardiacap II, Datex, UK) for 30 min and at 5-min intervals for additional 30 min after completion of the study. Tympanic membrane (core) and oral temperatures were measured with thermocouples having a rapid response and an accuracy range of ± 0.1 °C (Mon-A-Therm, Inc., St Louis, MO).

Hypotension was defined as a 20% reduction in arterial pressure in relation to baseline or a systolic pressure less than 100 mm Hg.

Chi-square analysis was performed for frequency

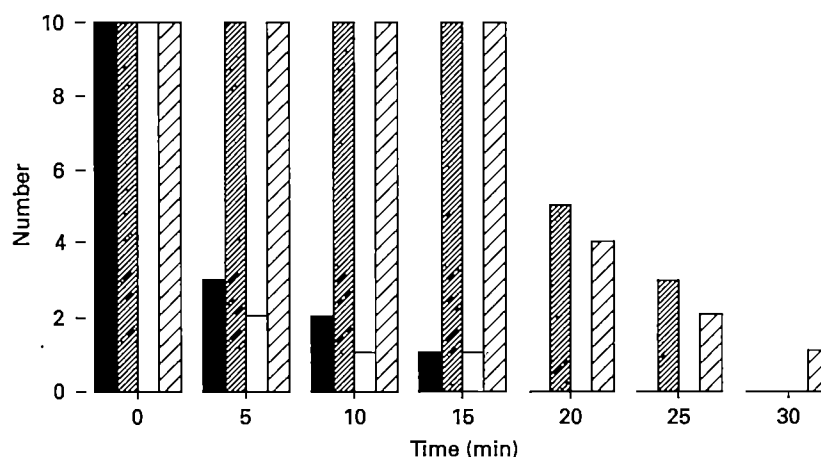


FIG. 1. Number of patients with shivering before (time 0) and after the administration of the study solutions. Patients received i.v. clonidine 30 µg or saline. If after this initial bolus shivering was not suppressed, the therapy (clonidine or saline) was repeated every 5 min to a maximum of three boluses. After 15 min, patients who received clonidine were given saline and *vice versa* with the same procedure. Group I = Clonidine/saline (■ = Caesarean section; □ = vaginal delivery). Group II = Saline/clonidine (■ = Caesarean section; ▨ = vaginal delivery).

data and Student's paired and unpaired sample *t* tests were used for normally distributed variables. $P < 0.05$ was considered statistically significant.

All the patients were normotensive and complained of shivering and discomfort requiring therapy before administration of the study solutions.

The administration of clonidine inhibited shivering after 15 min in all patients, and in 75 % of them, shivering ceased within 5 min after only one dose of clonidine 30 µg. In contrast, none of the patients treated with saline improved. When patients in the placebo group received clonidine there was an improvement (fig. 1).

In the group of patients who received saline before clonidine, there was a non-significant trend towards a greater total dose of clonidine necessary to suppress shivering.

There were no significant changes in AP, HR, core and peripheral temperature and oxygen saturation during the study between and within the groups before and after administration of clonidine. None of the patients receiving a single bolus of clonidine 30 µg had hypotension. One patient who received a total dose of clonidine 90 µg had moderate hypotension which responded to i.v. fluids. This patient belonged to the Caesarean section group and had, at the time of hypotension, a sensory level of analgesia above T2.

COMMENT

Clonidine is an α_2 -adrenoceptor agonist used as an antihypertensive drug. It possesses potent spinal and central antinociceptive properties. Clonidine has many effects on the central nervous system, including a reduction in body temperature.

Other workers observed a reduction in the number of episodes of postoperative shivering after continuous infusion of clonidine 7 µg kg⁻¹ over 120 min in patients undergoing aortic surgery [3]. The intraoperative administration of a continuous infusion of clonidine 5 µg kg⁻¹ over 3 h reduced oxygen uptake and the intensity of mean muscular tremor after major abdominal surgery [4]. A single

bolus of clonidine 150 µg i.v. inhibits postoperative shivering after general or extradural anaesthesia in 90–95 % of patients [1, 2]. Unfortunately, the administration of such a large dose may be associated with a significant decrease in mean arterial pressure [1, 2].

In our study, in the majority of patients we gave only 30 µg of clonidine and this dose was sufficient to suppress shivering without any haemodynamic change. The mechanism of action of such a rapid suppression of shivering after i.v. administration of a small dose of clonidine is not known.

Experimentally, hypothalamic thermoregulatory effects have been demonstrated for noradrenergic agonists such as clonidine [5]. Clonidine may exert an inhibitory action on the hypothalamus by decreasing noradrenaline synaptic release through α_2 receptors located at presynaptic axon terminals [6].

Our preliminary findings suggest that low-dose i.v. clonidine may be useful in abolishing severe post-extradural shivering in parturients. Larger studies are needed to evaluate the benefits of this method.

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CASE REPORTS

DELAYED SURGICAL EMPHYSEMA, PNEUMOMEDIASTINUM AND BILATERAL PNEUMOTHORACES AFTER POSTOPERATIVE VOMITING

W. G. M. BREMNER AND C. M. KUMAR

SUMMARY

We describe a case of surgical emphysema, pneumomediastinum and bilateral pneumothoraces which occurred some hours after general anaesthesia for a repeat laparoscopy and followed persistent nausea and vomiting. We report the case because of the unexpected and delayed appearance, which led to delay in diagnosis and management. We suggest that this intrathoracic air leak was a consequence of postoperative vomiting rather than a complication of laparoscopy. (Br. J. Anaesth. 1993; 71: 296–297)

KEY WORDS

Complications: nausea, vomiting, surgical emphysema, pneumomediastinum.

Although surgical emphysema, pneumomediastinum and pneumothorax are well recognized complications of laparoscopic procedures, they usually become manifest during or immediately after operation [1–4]. The delayed development of these complications has not been described after general anaesthesia associated with postoperative vomiting.

CASE REPORT

A 37-yr-old woman under investigation for pelvic pain presented for a third laparoscopy in 4 years. She had undergone two previous laparoscopies which were uneventful except for postoperative nausea and vomiting. She weighed 55 kg, did not smoke and was otherwise healthy.

She was premedicated with temazepam 20 mg and metoclopramide 10 mg orally 1 h before operation. An induction sequence of fentanyl 75 µg, propofol 150 mg and atracurium 20 mg was followed by atraumatic tracheal intubation with a cuffed, 8-mm oral tube. Breath sounds were auscultated and ventilation (Manley Pulmovent MPP) to normocapnia achieved with a fresh gas flow of 7 litre min⁻¹. Tidal volume was 600 ml and peak airway pressure 18 cm H₂O. Anaesthesia was maintained with 1% enflurane and 66% nitrous oxide in oxygen. Monitoring included electrocardiography, automated arterial pressure (Dinamap), pulse oximetry, capnography (Normocap, Datex), airway pressure, expired tidal volume (Wright Respirometer) and a nerve stimulator (Bard).

A pneumoperitoneum was formed with ease by the insufflation of carbon dioxide 3 litre and the insufflator pressure alarm was set at 15 mm Hg. After 20 min of laparoscopy (no intervention), the pneumoperitoneum was released and residual neuromuscular block antagonized with neostigmine 2.5 mg and glycopyrronium 0.5 mg. There were no problems associated with artificial ventilation during the procedure. The patient's trachea was extubated uneventfully after return of spontaneous ventilation. She was transferred to the recovery room, administered oxygen 4 litre min⁻¹ (Hudson mask) and routine clinical observations were satisfactory (heart rate, arterial pressure, oxygen saturation and ventilatory frequency). Morphine 15 mg and metoclopramide 10 mg were administered i.m. for abdominal pain in the recovery area. The patient complained of nausea and vomited several times.

Three hours after transfer to the ward, the patient complained of central chest pain, tightness across her chest and mild dyspnoea. Surgical emphysema restricted to her face and neck developed. An urgent chest radiograph revealed subdiaphragmatic gas and subcutaneous emphysema, but no pneumothorax. Conservative management involved reassurance, analgesia and antiemetic and oxygen therapy. There was no further vomiting. The chest discomfort and tightness continued overnight. A chest radiograph on the first day after operation revealed mediastinal emphysema and bilateral pneumothoraces, in addition to the pneumoperitoneum and subcutaneous emphysema. The larger pneumothorax was less than 25% of the lung field. Chest drains were not inserted. After 4 days of observation during which the subcutaneous emphysema and pneumothoraces resolved slowly, the patient was discharged home. At review 1 month later she was well with a normal chest radiograph and normal lung functions tests.

DISCUSSION

Several mechanisms associated with laparoscopy may result in the abnormal presence of gas in the subcutaneous tissue, mediastinum and pleural cavity. Local subcutaneous emphysema may arise if

carbon dioxide is insufflated too superficially because of incorrect positioning of the Verres needle [3]. One case of subcutaneous emphysema of the anterior chest wall has been attributed to inaccuracy of the intra-abdominal pressure gauge on the carbon dioxide insufflator machine resulting in an unintentionally high intra-abdominal pressure [2]. Pneumomediastinum may be produced by cephalad dissection and tracking of gas insufflated either preperitoneally or retroperitoneally through an unintentional peritoneal tear [3]. Mediastinal gas may then rupture through the mediastinal pleura to cause a pneumothorax. Pneumothoraces may result also from the transdiaphragmatic passage of insufflating gas through an acquired or congenital pleuroperitoneal defect [5-7]. However, it is anticipated that the problems caused by the passage of insufflated gas (carbon dioxide) during operation into the extraperitoneal area are transient because of the relatively great solubility of carbon dioxide [3, 8].

Although mediastinal emphysema may be spontaneous in the absence of underlying disease, it occurs most frequently in one of several situations which have in common an increase in alveolar pressure such as sneezing, straining, the Valsalva manoeuvre and protracted vomiting [9]. Other possible causes of this patient's pathology not related to gas insufflation include trauma, penetrating injury or damage during instrumentation to the pharynx, larynx, trachea and oesophagus [9, 10] and pulmonary barotrauma [11]. However, intubation was atraumatic, airway pressure not excessive and the patient did not develop the serious clinical sequelae of an oesophageal tear.

The most common cause of mediastinal emphysema is alveolar rupture [9], but for this to occur, the pressure within the alveolus has to exceed that in the surrounding tissue and air from the ruptured alveoli then tracks along the bronchovascular sheaths to the lung hila and mediastinum, whence it may spread along fascial planes to cause subcutaneous emphysema in the face, neck, chest or abdomen [9]. However, if the rate of escape of air from mediastinum is insufficient, the mediastinal pleura may rupture to cause pneumothorax [12, 13]. In our patient, we believe that there was an excessive increase in alveolar pressure after vomiting, leading to alveolar rupture, and that the gas took the line of least resistance along the fascia surrounding the bronchovascular tree to reach the mediastinum [14, 15]. It seems likely that the offending gas was oxygen-enriched air, so that subcutaneous emphysema, mediastinal emphysema and pneumothoraces were slow to develop and resolve because of equilibration of gases with blood [16]. However, we

did not obtain any samples which might have revealed a reduced carbon dioxide tension.

We conclude that subcutaneous emphysema, mediastinal emphysema and pneumothorax may develop after postoperative vomiting. There may be no obvious associated factor and the complete picture may not develop in the immediate postoperative period. A case of delayed subcutaneous emphysema after operation should be investigated intensively and the surgical procedure, such as laparoscopy, should not always be assumed to be the cause.

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PULMONARY EMBOLUS AND PATENT FORAMEN OVALE: A RARE CAUSE OF REFRACTORY HYPOXAEMIA

C. BRYDON, W. J. FAWCETT, T. TREASURE AND J. T. CLARKE

SUMMARY

A patient with a large pulmonary embolus is described. The striking features were those of cardiovascular stability with hypoxaemia and hypercapnia. Cardiac catheterization confirmed the pulmonary embolus; in addition there was found to be a patent foramen ovale (PFO), causing a large right-to-left shunt. Removal of the pulmonary embolus and closure of the PFO dramatically improved alveolar-arterial oxygen gradient and ventilatory requirements. (Br. J. Anaesth. 1993; 71: 298–300)

KEY WORDS

Oxygen: hypoxaemia. Heart: patent foramen ovale. Complications: pulmonary embolism.

CASE REPORT

A 64-yr-old man collapsed in the street, and was asystolic on arrival at the local Accident and Emergency Department. Protracted attempts at resuscitation resulted in the restoration of sinus rhythm, and an arterial pressure of 130/80 mm Hg. He remained hypoxaemic, with a P_{aO_2} 7–10 kPa, with the trachea intubated and the lungs ventilated with 100% oxygen. The previous day he had visited his General Practitioner complaining of unilateral calf pain; in view of this history, a pulmonary embolus was suspected, and administration of streptokinase 1.5 mu was commenced. He was transferred to the regional cardiothoracic centre for further investigation and management.

On arrival, the patient was deeply unconscious. Physical examination revealed bilateral air entry to both lung fields. Arterial pressure was 140/80 mm Hg, heart rate 120 beat min⁻¹ and right atrial pressure (RAP) 4 mm Hg. There were no murmurs detectable on auscultation. The only other abnormality was a swollen right calf. Chest x-ray was unremarkable and the ECG showed sinus rhythm with no signs of right heart strain. He had been oliguric for several hours.

The absence of hypotension with normal RAP, together with no ECG evidence of right heart strain conflicted with the diagnosis of pulmonary embolism and yet the patient remained both hypoxaemic and hypercapnic. Arterial blood-gas analysis revealed P_{aO_2} 9.1 kPa, and P_{aCO_2} of 8.3 kPa during ventilation with 100% oxygen and 5 cm H₂O of positive end-

expiratory pressure (PEEP) with a minute volume of 17 litre. After discussion with the admitting cardiologist and cardiac surgeon, it was decided to clarify the situation by cardiac catheterization. The explanation was immediately evident, as the right atrial catheter passed through a patent foramen ovale (PFO). Large right and left proximal pulmonary emboli were also noted. Left ventricular function and coronary arteries were normal. His outlook seemed extremely poor without intervention, so he was therefore prepared for closure of his PFO, and pulmonary embolectomy.

On arrival in the operating theatre, the patient was receiving infusions of atracurium 40 mg h⁻¹ and midazolam 16 mg h⁻¹. He was given a bolus of fentanyl 1 mg. His lungs were ventilated with 100% oxygen, using an Oxylog ventilator with a minute volume of 16 litre. Arterial blood-gas tensions before cardiopulmonary bypass (CPB) were P_{aO_2} 10.7 kPa and P_{aCO_2} 6.56 kPa; pH was 7.26.

On bypass, the right atrium was opened. It was noted by the surgeon that the PFO had an intact, valve-like mechanism, preventing passage of blood from left to right atrium until the floor of the fossa ovalis was deflected. In the presence of increased right atrial pressure, this would have permitted a large right-to-left shunt. The PFO was sutured, the pulmonary artery opened and considerable amounts of thrombus removed from each side.

The myocardial ischaemic time was 5 min and CPB was discontinued without difficulty after 30 min. Within 15 min there was a dramatic improvement in gas exchange. With a minute volume of 7 litre and FI_{O_2} 1.0, arterial blood-gas tensions were P_{aO_2} 46.1 kPa and P_{aCO_2} 5.42 kPa; pH 7.25.

In spite of improvement in gas exchange, the patient developed acute renal failure requiring at first continuous and later intermittent haemodialysis. In addition, he remained deeply unconscious for nearly 2 weeks, suffering several grand mal fits. This was attributed to hypoxaemic brain damage at cardiac arrest. Thereafter there was slow but unequivocal neurological recovery.

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Three weeks after admission, he was transferred to the regional renal unit and diuresis commenced soon after transfer. At 3 months, he returned to an outpatient clinic, apparently neurologically intact and with near normal renal function (serum concentrations of urea $8.2 \text{ mmol litre}^{-1}$; creatinine $130 \text{ } \mu\text{mol litre}^{-1}$).

DISCUSSION

Pulmonary embolism is not uncommon—the incidence in the United States is reported as 600 000 per year [1]. In addition, up to 35 % of the population may have a probe-patent PFO [2], although only 5–10 % have a flow-patent PFO [3, 4]. The fact that these two conditions coexist is thus unsurprising, and when they do there is a greater incidence of adverse events and death compared with pulmonary embolism alone [5]. There are previous case reports of paradoxical emboli occurring as a result of venous thromboembolism and intracardiac shunts [4, 6–11]. It appears that more than 50 % of cardiac defects result from PFO, with pulmonary arteriovenous malformations and ventricular or atrial septal defects accounting for the remainder [11].

A large pulmonary embolism causes a dramatic increase in afterload to the right ventricle and, if the patient survives the acute event, there is persistent poor cardiac output and systemic hypotension. In this patient, the increase in right heart pressures caused blood to flow from right to left through the PFO. This permitted adequate left ventricular filling, which presumably maintained a near normal left ventricular output, but with desaturated arterial blood. Following surgery, there was a substantial improvement in gas exchange. In contrast with the patient reported here, one large study of pulmonary embolism with or without PFO found that the mean P_{aO_2} was only 1 kPa less in the embolism and PFO group compared with the embolism group alone [5]. This is difficult to explain if a large right-to-left shunt is present.

There are other mechanisms of hypoxaemia in pulmonary emboli, although hypoxaemia is by no means universal. There may be reflex bronchoconstriction [12] and pulmonary venous hypertension from platelet-derived serotonin, causing pulmonary oedema [13–14]. In addition, a reduction in cardiac output (with a corresponding reduction in mixed venous oxygen saturation) and inadequate ventilation of over-perfused lung, venoarterial shunting and, later, reperfusion of poorly ventilated lung zones may contribute to hypoxaemia [1]. In addition to the effects on arterial oxygen saturation, there is an increase in deadspace, secondary to obliteration of the pulmonary vascular bed.

Surgery for pulmonary embolism is contentious [15]. The large majority of victims of acute massive pulmonary embolism die within minutes, or recover without major cardiac surgery. Nevertheless, there are a few in whom surgery has a place, when thrombolysis is ineffective or contraindicated. Gray and colleagues have reviewed pulmonary embol-ectomy for acute massive pulmonary embolism in 71 patients over 22 years [16]. All patients were haemodynamically severely compromised; 30 % of

them died and, in particular, the death rate was much greater in those who had received external cardiac massage compared with those who had not (64 % vs 11 %). The morbidity was principally neurological, thought to have occurred before surgery as a result of poor cerebral perfusion before operation. Our patient is of interest in that he suffered cardiac arrest when out of hospital and arrived asystolic (although we do not know for how long he was without a cardiac output). Following successful resuscitation, any cerebral insult that might have been sustained would have been compounded by both hypoxaemia and hypercapnia which continued for 10 h until corrected by surgery. Nevertheless, he was eventually left without neurological deficit. This emphasizes not only that resuscitation and surgery were fully justified, but that the decision to resuscitate should be based on the cardiovascular response to treatment, and not on neurological assessment made at the time of resuscitation.

In the majority of patients a PFO is of little significance. The valvular nature of the flap prevents blood flowing from left to right and, as the normal left atrial pressure (LAP) exceeds right atrial pressure (RAP), blood does not flow. In conditions in which this interatrial gradient is reversed, which may occur with any sudden increase in right ventricular afterload, right-to-left shunting may occur [3]. Recently, it has been demonstrated by transoesophageal echocardiography that intermittent positive pressure ventilation in ASA I patients caused right-to-left shunting in 9 % of patients when 10–15 cm H_2O of PEEP was applied [4]. Thus right-to-left shunting through a PFO should be considered in any patient in whom RAP may exceed LAP, and who becomes hypoxaemic. In our patient, PEEP was used before operation: this may well have contributed to the magnitude of the right-to-left shunt.

End-tidal P_{CO_2} ($P_{E'CO_2}$) measurement would have been of interest in this patient, both for monitoring and for diagnosis, although the urgency of the situation did not permit detailed recordings. $P_{E'CO_2}$ is reduced in acute pulmonary embolism secondary to the reduction in pulmonary blood flow, and a large arterial– $P_{E'CO_2}$ gradient has been described as a sensitive indicator for pulmonary embolism compared with other causes of ventilation–perfusion mismatch [17]. However, in the presence of a clinically significant cardiac output, a large arterial– $P_{E'CO_2}$ gradient would have suggested the co-existence of a right-to-left shunt. Continued monitoring of the gradient could have been used to assess the effects of intervention or to detect new emboli and, indeed, has been suggested for this purpose [18].

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MULTIPLE CORONARY ARTERY DISSECTIONS IN A WOMAN AT TERM

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SUMMARY

We report a case of acute myocardial infarction caused by multiple coronary artery dissections in a pregnant woman at term. When reinfarction elicited fetal distress, Caesarean section was performed under carefully administered extradural anaesthesia, with maternal and fetal survival. (Br. J. Anaesth. 1993; 71: 301–302)

KEY WORDS

Anaesthesia: obstetric. Heart: ischaemia, myocardial infarction.

CASE REPORT

A 35-yr-old gravida 1, 40 weeks pregnant, was admitted to the coronary care unit with substernal pain radiating to the left arm. There was no family history of cardiovascular disease, but the patient was overweight (118 kg, 170 cm) and smoked 20 cigarettes per day. She was pale and dyspnoeic, with an arterial pressure of 140/90 mm Hg, a regular heart rate of 110 beats min⁻¹, and slight pitting oedema. A third heart sound was heard without murmurs; breathing was normal. The ECG demonstrated transmural anteroseptal myocardial infarction, but chest x-ray was normal. Serum concentrations of creatinine phosphokinase (CK), CK-MB and α -hydroxybutyrate dehydrogenase (α -HBD) were increased (CK 1154 u litre⁻¹, CK-MB 101 u litre⁻¹, α -HBD 459 u litre⁻¹). Other laboratory values, including lipid profile, were within normal limits; there was no proteinuria. The symphysis–fundal height was 38 cm; the fetus, in the vertex position, had a reactive heart rate pattern; the cervix was 1 cm dilated.

Treatment consisted of continuous i.v. infusion of nitroglycerin 30 μ g min⁻¹ and heparin 25 000 iu/24 h. On the third day, the patient experienced renewed chest pain, the ECG showed recurrent (infero-posterior) infarction and concentrations of cardiac enzymes increased further (CK 2300 u litre⁻¹, CK-MB 214 u litre⁻¹ and α -HBD 1112 u litre⁻¹); the haemoglobin concentration decreased from 7.2 to 6.6 mmol litre⁻¹. A thermodilution catheter was introduced via the right brachial vein. Cardiac output and arterial pressures were normal (7.6 litre min⁻¹, 120/80 mm Hg, respectively); heart rate, pulmonary artery pressures and pulmonary capillary wedge pressure were increased (135 beat min⁻¹,

43/32 mm Hg and 28 mm Hg, respectively); pulmonary and systemic vascular resistances were reduced or normal (36 and 898 dyn s cm⁻⁵, respectively). Fetal heart rate showed loss of variability, and a decision to perform Caesarean section was made. The rate of infusion of nitroglycerin was increased to 50 μ g min⁻¹, frusemide 40 mg was given and the heparin infusion was discontinued. An extradural catheter was introduced at the L3–4 interspace and 0.25 % bupivacaine 6 ml was injected. Anaesthesia was increased slowly with five 3-ml aliquots of 0.5 % bupivacaine with fentanyl 5 μ g ml⁻¹ in 60 min. During this time, fetal heart rate variability returned to normal (fig. 1). As the extradural block developed, Ringer's lactate 1000 ml was infused. Maternal systemic arterial pressure remained relatively stable, while pulmonary pressures decreased towards normal values (fig. 2). An uncomplicated Caesarean section was performed when the block had reached T4. A healthy 3400-g girl was delivered with 1 and 5 min Apgar scores of 9 and 10, respectively, and an umbilical artery pH of 7.29. After clamping of the cord, oxytocin was infused at a rate of 2.5 mg h⁻¹. Blood loss during surgery was estimated at 600 ml, therefore 2 units of packed cells were given. After completion of the procedure, the patient received an additional 40 mg of frusemide.

Coronary angiography the following day showed dissections in all three coronary arteries. Echocardiography showed a dilated left ventricle with hypokinetic apical and infero-posterior regions. Despite unchanged angiographic findings, 1 month later the patient had an exercise performance of 140 W, under medication. After 6 months she still requires medication: digoxin, enalapril, isosorbide-5-mononitrate, acetosalicylic acid and acenocoumarol; she does not smoke and is on a low-salt diet.

DISCUSSION

Myocardial infarction in a term pregnant woman is a rare event and carries a high mortality. Fifty percent of 84 cases reported since 1922 occurred in the third

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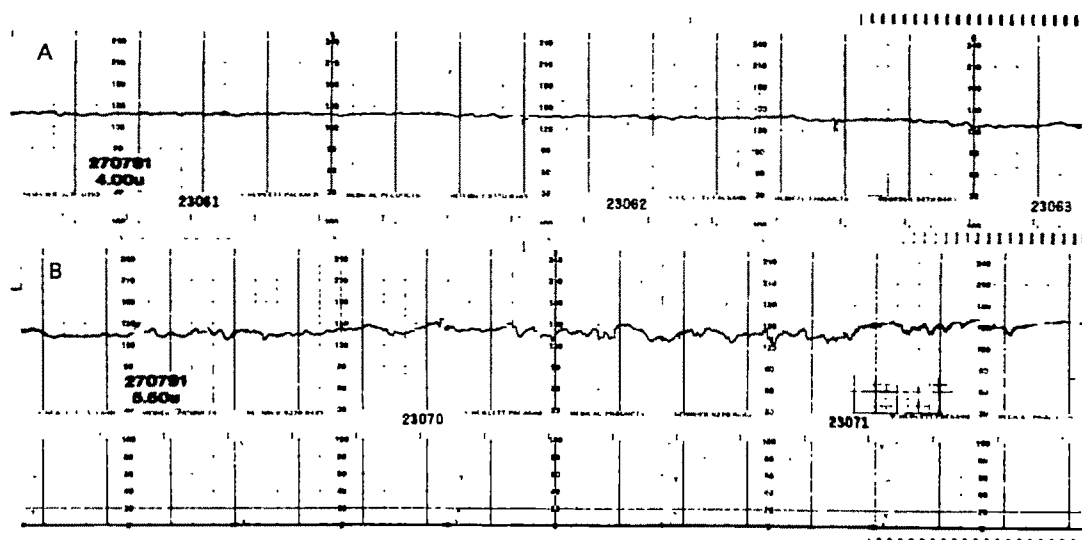


FIG. 1. Fetal heart rate pattern. A: Limited variability of fetal heart rate, suggestive of hypoxaemic distress, after maternal myocardial reinfarction. B: Normal variability of the fetal heart rate, suggestive of improvement of hypoxaemic distress, after extradural anaesthesia.

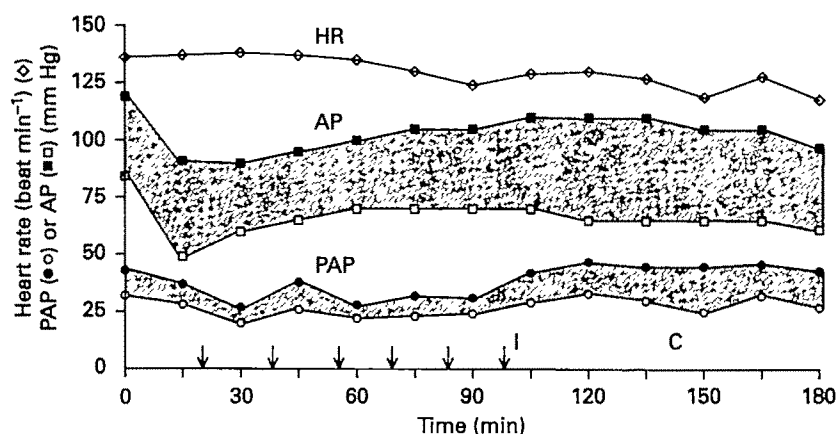


FIG. 2. Some cardiovascular variables during Caesarean section under extradural anaesthesia in a term pregnant woman with myocardial reinfarction. Hypotension at 15 min resulted from increased rate of infusion of nitroglycerin. Extradural anaesthesia was performed with small aliquots of bupivacaine and fentanyl to assure haemodynamic stability. $\diamond$ = Heart rate; $\blacksquare$ = systolic arterial pressure, $\square$ = diastolic arterial pressure; $\bullet$ = systolic pulmonary arterial pressure, $\circ$ = diastolic pulmonary artery pressure. $\downarrow$ = aliquot of extradural anaesthetic; I = abdominal incision; C = abdominal closure.

trimester, with a mortality of 40%; the mortality in the first two trimesters was 20% [1]. Three of four reported women with myocardial reinfarction died. A diagnosis of dissecting coronary aneurysm was made in two pregnant women at postmortem [2]. The present patient demonstrated that dissecting coronary aneurysm in pregnancy is not invariably fatal.

Our patient was initially managed optimistically to avoid the haemodynamic changes associated with surgery. When fetal distress demanded Caesarean section under the unfavourable condition of recurrent myocardial infarction, maternal haemodynamic stability was maintained throughout the carefully performed extradural anaesthesia and Caesarean section. The improvement in fetal heart rate variability most likely resulted from improved uterine blood flow, which is known to occur under controlled sympathetic block. The standard procedure of preloading with 1–2 litre of crystalloids before the development of extradural block could not be used because the patient was at the limit of

decompensation. Instead, by fine tuning of fluid balance and intravascular capacity using nitroglycerin, frusemide and the developing sympathetic block, while at the same time anticipating the autotransfusion from the retracting uterus after delivery, haemodynamic stability could be maintained.

This case report demonstrates that Caesarean section under extradural anaesthesia is a safe and valuable procedure, even in the unfavourable condition of acute myocardial reinfarction caused by coronary artery dissection.

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APPARATUS

FLOW REVERSAL THROUGH A MARK III HALOTHANE VAPORIZER

A. S. GREGG, R. S. JONES AND S. L. SNOWDON

SUMMARY

A fault in the assembly of a Matrix Large Animal Circle anaesthetic machine resulted in reversal of fresh gas flow through the vaporizer. The fault was discovered only after the sudden development of excessive depth of anaesthesia in two equine patients. Laboratory investigations were conducted to determine the effect of flow reversal on vaporizer output. Results indicated that output concentration was approximately doubled under these conditions. (Br. J. Anaesth. 1993; 71; 303–304)

KEY WORDS

Equipment: vaporizer, reverse flow.

The effects of intermittent back pressure on vaporizer function have been reported extensively. Hill and Lowe found that concentrations delivered by susceptible vaporizers during controlled or assisted ventilation were considerably greater than when the vaporizer was used with free flow [1]. The effect is greatest at small gas flows [2]. However, little information has been published on the potential hazards of reversing the fresh gas flow rate through the vaporizer. Marks and Bullard concluded that flowing gases in a reverse direction resulted in approximately double the output indicated on the vaporizer dial [3].

This paper describes inadvertent reversal of flow through a vaporizer used for anaesthesia in two equine patients. When the fault had been discovered and rectified, the vaporizer was subjected to laboratory investigation.

CASE REPORTS

Two horses required general anaesthesia for orthopaedic surgery, the first for an elective procedure and the second for emergency fracture repair. Preanaesthetic examination revealed no evidence of concurrent disease.

All anaesthetic equipment was checked by the anaesthetist before use and the standard anaesthetic practice of this hospital was followed in each case. Cardiovascular and ventilatory variables were monitored by methods currently accepted in this species [4, 5] and the vaporizer setting adjusted accordingly. Despite this, in each of the animals there was a sudden deterioration in cardiovascular

and respiratory status, and a loss of ocular reflexes after being moved to the operating table. The cause of this dramatic increase in depth of anaesthesia was not apparent at the time.

Appropriate resuscitative therapy was successful in each case. The animal undergoing the elective procedure was allowed to recover immediately after resuscitation without surgical intervention. Subsequent investigation revealed that the fresh gas flows had been routed inadvertently through the vaporizer in the reverse direction. The apparatus involved was a new Large Animal Circle (Matrix) with a Fluotec Mark III vaporizer which had not been used previously. Two other large animal anaesthetic machines, including a mechanical ventilator (North American Dräger) have been in regular use at the hospital for several years. Although the incidents occurred 2 weeks apart, this particular anaesthetic machine was not used in the intervening time.

Laboratory investigation

Halothane vapour concentration was measured using an on-line organic anaesthetic analyser (Wetenschappelijk Technische Instrumentatie B.V. Anaesthetic Gas Analyser (W.T.I.)). This system uses a specially designed hydrogen flame-ionization detector (FID) [6]. It is sensitive only to organic agents, in this case the anaesthetic used; inorganic components, such as oxygen, are not detected. The ionization current, measured with an electrometer amplifier, is proportional to the amount of organic anaesthetic agent that reaches the FID per unit time. Step response-time is approximately 0.2 s, and the small dimension of the capillary transport tube ensures that there is no mixing between successive samples. The large dynamic range, excellent stability and high sensitivity of this detector are well known from gas chromatography applications [7].

The unit was first calibrated using a W.T.I. Anaesthetic Gas Calibrator, designed specifically for this purpose [7, 8].

The first part of the investigation involved measurement of vaporizer output with the flow in the forward direction. The vaporizer (Fluotec Mark

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TABLE I. *Halothane concentrations delivered at various gas flow settings during normal and reversed flows*

Flow rate (litre min ⁻¹)(%)	Dial setting Normal flow	Delivered concentration (%)	
		Reverse flow	
1	1	0.9	1.25
	2	1.75	2.40
	3	2.9	3.95
	4	4.0	5.05
	5	4.9	6.0
4	1	1.0	1.6
	2	2.0	3.3
	3	3.2	5.05
	4	4.1	6.1
	5	4.85	8.05
8	1	0.75	2.1
	2	1.6	4.2
	3	2.6	6.6
	4	3.45	7.6
	5	4.0	8.2

III) was connected in a free-standing manner to a standard anaesthetic trolley (British Oxygen Company Ltd, BOC). A short length of corrugated tubing, which was bent to ensure adequate mixing of the vapour, was attached to the vaporizer outlet. The capillary transport tube from the W.T.I. analyser was placed into this tubing for sampling. The position and orientation of the sampling needle remained constant throughout the investigation. Results were displayed continuously using a chart-recorder.

Using an initial flowmeter setting of 1 litre min⁻¹, oxygen was delivered through the vaporizer in the forward direction. The vaporizer dial setting was adjusted in 1 % increments from 1 to 5 % halothane, and the output at each setting recorded.

The procedure was repeated using flowmeter settings of 4 litre min⁻¹ and then 8 litre min⁻¹. Each test was performed twice and mean values calculated (table I). The analyser was re-calibrated between successive tests to prevent drift.

The second part of the investigation involved measurement of vaporizer output with the flow in the reverse direction. For this purpose, the inlet and outlet pipes were transposed and the procedure repeated exactly as before.

Temperature and pressure remained constant (24 °C and 760 mm Hg, respectively).

As the purpose of this investigation was to demonstrate the grossness of the error, only two readings were taken at each vaporizer setting and a mean value calculated (table I). No attempt was made to define a calibration curve for the vaporizer under these conditions.

DISCUSSION

The anaesthetic machine standard requires that the inlet of a vaporizer be male and the outlet female, the direction of gas flow marked and the inlet and outlet parts labelled [9].

Whilst all these requirements were met in the Large Animal Circle (Matrix) used in both of these

anaesthetics, the rubber tubing designed to deliver fresh gases from the flowmeters to the vaporizer inlet and that leading from the vaporizer outlet to the breathing system are of the same appearance and diameter. In addition, hose connections had not been crimped by the manufacturers. These factors allow the possibility of incorrect assembly, with the gas flowing through the vaporizer in the reverse direction.

The American design of this particular circle system, with the vaporizer sited to the left of the flowmeters, is such that it may not be immediately obvious to the observer that such a mistake has been made. The machine was used exactly as it was supplied by the manufacturers and the routine preoperative equipment check did not reveal that our apparatus had been assembled incorrectly.

It cannot be over-emphasized that anaesthetists should be familiar with any piece of equipment before they attempt to use it, and that a thorough check of all apparatus is made before each use. However, we do feel that the design of this particular model might be modified in such a way as to make it impossible to connect the tubing to the wrong male/female connectors in this manner.

Marks and Bullard also drew attention to the fact that connecting a free-standing vaporizer in reverse is easily done and easily overlooked [3]. Where portable vaporizers are used, special care must be taken to ensure that gases flow through them in the correct direction because, although inlet and outlet sides of the vaporizer are marked, the labelling is not conspicuous and the same size of tubing fits each side.

ACKNOWLEDGEMENTS

We are grateful to Mr Martin Jones for technical assistance.

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ABSTRACTS

PROCEEDINGS OF THE ANAESTHETIC RESEARCH SOCIETY

NEWCASTLE UPON TYNE MEETING

April 2-3, 1993

(The name of the author presenting the paper is shown in bold type. *Indicates non-member.)

All authors have certified that, where appropriate, studies have been conducted with the approval of the relevant Human Ethics Committee or Animal Experimental Review Committee.

RELATIVE EFFECTS OF CLONIDINE ON A δ AND C REFLEXES AND SPONTANEOUS SYMPATHETIC ACTIVITY

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Clonidine, an α_2 adrenoceptor agonist, inhibits nociceptive reflexes [1] and causes cardiovascular depression [2]. Opioids and midazolam injected intrathecally (i.t.) cause greater depression of reflexes evoked by C and A δ fibres, respectively, without measurable effects on local efferent sympathetic activity [3]. We investigated the selectivity of the effect of clonidine.

Experiments were performed on eight anaesthetized, paralysed and artificially ventilated dogs. Anaesthesia was induced with methohexitone 15 mg kg⁻¹ and maintained with 1% α -chloralose 30 mg kg⁻¹, followed by an infusion of 10-20 mg kg⁻¹ h⁻¹. The dogs' lungs were intubated and muscle paralysis was achieved with suxamethonium 10 mg every 30 min. The lungs were ventilated with oxygen-enriched air and temperature and blood-gas tensions were maintained within physiological limits. Radial, tibial and renal sympathetic nerves were exposed, desheathed, cut distally and mounted on silver-silver chloride electrodes in mineral oil pools. The dura was exposed by laminectomy in four dogs to inject drugs i.t. Spontaneous renal sympathetic nerve activity was amplified, displayed and integrated. Supramaximal electrical stimuli (30 V, 0.5 ms, 0.33 Hz) were applied to the peripheral nerves. The reflex renal sympathetic nerve responses were displayed, averaged (16 responses), rectified and integrated. Four dogs received clonidine i.t. in doses of 50 μ g, 100 μ g and 150 μ g in 0.5 ml, 1 ml and 1 ml, respectively, at 15-min intervals. The other four dogs received clonidine i.v. in doses of 50 μ g, 100 μ g, 150 μ g and 300 μ g at 15-min intervals. Naloxone 2 mg i.v. was administered 15 min after the last dose of clonidine followed by the α_2 adrenoceptor antagonist yohimbine 5 mg i.v. at 15-min intervals until measurements had returned to control values.

Incremental doses of clonidine i.t. caused non-selective, dose-dependent depression of A δ and C reflexes from tibial nerve stimulation, which were abolished by a cumulative dose of 150 μ g. At this point there was a reduction in spontaneous renal sympathetic nerve activity of 80% and no reduction in radial evoked responses. With clonidine 300 μ g, although spontaneous renal sympathetic nerve activity was also abolished, there was only a small reduction in radial evoked responses. In contrast, i.v. administration caused progressive similar reductions in spontaneous renal sympathetic nerve activity and both radial and tibial A δ and C reflexes, so that all were abolished simultaneously (cumulative dose 600 μ g i.v.). Clonidine 300 μ g i.t. caused a reduction in mean heart rate from 138 to 90 beat min⁻¹ ($P < 0.05$) and from 132 to 77 beat min⁻¹ ($P < 0.05$) after i.v. administration, which decreased further to 62 beat min⁻¹ with 600 μ g i.v. In contrast, there were only small reductions in mean arterial pressure (ns). The effects of clonidine were antagonized by yohimbine but not naloxone.

In conclusion, clonidine caused similar dose-dependent depression of A δ and C reflexes. When these were eliminated (150 μ g i.t.), 20% of local efferent sympathetic activity remained,

which was abolished by greater doses (300 μ g i.t.). In spite of this, the sympathetic responses to radial nerve stimulation remained unchanged indicating activation of a second non- α_2 descending efferent pathway. Systemic administration (600 μ g i.v.) caused simultaneous elimination of A δ and C responses to both tibial and radial stimulation and spontaneous efferent sympathetic activity. The effects of clonidine were antagonized by yohimbine but not by naloxone. The bradycardic effects of clonidine were confirmed.

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EFFECT OF LOW-DOSE ASPIRIN ON BLEEDING TIMES AND THROMBELASTOGRAPHY IN PATIENTS AT RISK OF PREGNANCY-INDUCED HYPERTENSION

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COMPARISON OF THE EFFECTS OF PREOPERATIVE NABILONE OR METOCLOPRAMIDE ON POSTOPERATIVE NAUSEA AND VOMITING

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Nabilone is a synthetic cannabinoid, used previously as an antiemetic during chemotherapy but not during anaesthesia [1]. After obtaining written informed consent and Institutional Ethics Committee approval, 60 patients undergoing total abdominal hysterectomy were randomized to receive oral premedication with either nabilone 2 mg or metoclopramide 10 mg, together with diazepam 10 mg. Anaesthesia comprised thiopentone and suxamethonium followed by pancuronium. Anaesthesia was maintained with isoflurane and nitrous oxide in oxygen. Papaveretum (total 0.3 mg kg⁻¹ i.v.) was given for analgesia, supplemented with diclofenac 100 mg as required, before recovery. Neuromuscular block was antagonized at the end of operation with neostigmine and glycopyrronium. Papaveretum 0.3 mg kg⁻¹ and prochlorperazine 12.5 mg were prescribed as necessary for postoperative analgesia and antiemesis. Nausea, vomiting, antiemetic and analgesic requirements were recorded both in the recovery area and on the ward. Patients were interviewed 24 h after operation by a researcher blinded to the premedication used, and were asked to score postoperative nausea, vomiting and pain using a 10-cm

visual analogue scale. They were also asked about sedation, relaxation and any side effects after premedication. Data were analysed using the Student's unpaired *t* test for parametric data, the Mann-Whitney *U* test for non-parametric data and the chi-square test with Yates' correction for non-continuous data.

Fifty-three patients completed the study; 27 received metoclopramide, 26 nabilone. The two groups were comparable for age and weight (table I). No significant differences were observed between the two groups with regard to nausea, vomiting, pain score or time to first oral fluids. The nabilone group received less opioid in recovery (*P* = 0.03), although no difference was seen in analgesic requirement between the two groups on the ward. Side effects were minimal; one patient receiving metoclopramide complained subsequently of dizziness.

We conclude that, with the dose used, nabilone is no more effective than metoclopramide.

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ANTICHOLINERGIC PREMEDICATION AND POSTOPERATIVE SHIVERING

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Postoperative shivering is a common phenomenon. A previous study [1] suggested that anticholinergic premedication increases significantly the incidence of postoperative shivering. This prospective, randomized, controlled study examined the effect of two different anticholinergic drugs, as premedication, upon the incidence and severity of postoperative shivering.

Two hundred and twenty-five healthy adult patients scheduled for ENT, dental or orthopaedic surgery were allocated randomly to receive one of three different premedications, given i.m. 1 h before operation: group 1 (*n* = 75) received morphine 0.15 mg kg⁻¹ and metoclopramide 10 mg; group 2 (*n* = 75) received morphine 0.15 mg kg⁻¹ and glycopyrronium 5 µg kg⁻¹ (maximum 400 µg); group 3 (*n* = 75) received morphine 0.15 mg kg⁻¹ and hyoscine 5 µg kg⁻¹ (maximum 400 µg). Patients received a standard anaesthetic technique, comprising i.v. induction with thiopentone 4-5 mg kg⁻¹ and maintenance with isoflurane in oxygen and nitrous oxide (*F*_IO₂ 0.3), breathing spontaneously via a Lack system. The duration of anaesthesia was recorded. In the recovery unit, patients were observed for the

TABLE II. Incidence (No.) and grade of postoperative shivering in the three groups of patients

Shiver grade	Group 1 (<i>n</i> = 75)	Group 2 (<i>n</i> = 75)	Group 3 (<i>n</i> = 75)
0	59	42	47
1	6	9	9
2	7	10	9
3	3	13	10

occurrence of shivering and its severity was graded (0 = none; 1 = mild fasciculation of the face or neck; 2 = visible tremor involving more than one muscle group; 3 = generalized shaking). Other variables recorded included heart rate, arterial pressure, ventilatory frequency, oxygen saturation and tympanic membrane temperature. Statistical analysis was performed using ANOVA, chi-square and Kruskal-Wallis tests as appropriate, using the SPSS-PC+ package.

There were no differences among the three groups for age, gender, weight or duration of anaesthesia. Patients who received anticholinergic premedication had a significantly greater frequency of postoperative shivering (grade > 0) and significantly greater shivering grades (Kruskal-Wallis, *P* < 0.05) than those in the control group (table II). There was no difference in core temperature between patients who shivered and others, either before or during the shivering episode. Shivering did not cause any significant changes in heart rate, arterial pressure, ventilatory frequency or oxygen saturation.

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EFFECT OF PROPOFOL ON PROPRANOLOL METABOLISM BY HUMAN LIVER MICROSOMES

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Mouton-Perry and colleagues [1] showed that continuous i.v. infusion of propofol 0.8 mg kg⁻¹ min⁻¹ decreased the intrinsic clearance of propranolol by 40% in the dog, implying that propofol inhibits the enzyme(s) responsible for the metabolism of propranolol. Propranolol undergoes aromatic hydroxylation in the 4 and 5 positions, side chain oxidation to the N-desisopropyl product, as well as direct O-glucuronidation. Propofol is also

TABLE I. Patient characteristics (mean (SD) or range), nausea, vomiting, antiemetic and analgesis requirements (% of group) in the recovery area and on the ward, and pain scores (median (range)) in the two groups

	Metoclopramide group	Nabilone group	<i>P</i> (test)
Age (years)	41.2 (26-54)	43.8 (23-67)	0.24 (<i>t</i> test)
Weight (kg)	74.3 (17.5)	65.1 (16.5)	0.15 (<i>t</i> test)
Recovery area			
Nausea (%)	29.6	23.1	0.82 (chi-square)
Vomiting (%)	22.2	15.4	0.50 (chi-square)
Antiemetic required (%)	18.6	19.2	0.50 (chi-square)
Opioid required (%)	51.9	19.2	0.03 (chi-square)
Ward			
Nausea (%)	70.4	73.1	0.93 (chi-square)
Vomiting (%)	66.7	53.8	0.50 (chi-square)
Antiemetic required (%)	70.3	57.7	0.50 (chi-square)
Opioid required (%)	92.6	100	0.49 (chi-square)
Time to oral fluid (h)	11.2 (6.0)	10.4 (6.7)	0.65 (<i>t</i> test)
Premedication			
Relaxed preop. (%)	96.3	92.3	0.97 (chi-square)
Sleepy preop. (%)	48.1	76.9	0.06 (chi-square)
Visual analogue scales			
Nausea	3.5 (0-10)	3.0 (0-7)	0.92 (<i>U</i> test)
Vomiting	3.0 (0-9)	1.5 (0-8)	0.32 (<i>U</i> test)
Pain	5.0 (0-10)	4.0 (0-6)	0.31 (<i>U</i> test)

metabolized by both oxidation and glucuronidation [2]. We have shown previously that propofol inhibits the conjugation of propranolol but not oxidation, in rat and dog liver microsomes [3]. We now report results using human liver microsomes.

Propranolol 50 $\mu\text{mol litre}^{-1}$ was incubated with human liver microsomes, fortified with either an NADPH-generating system or UDPGA. The incubations were performed at 37 °C in a shaking water bath for 45 min in the absence and presence of propofol dissolved in propylene glycol (0.5–28 $\mu\text{mol litre}^{-1}$). A control incubation containing propylene glycol was included. Each experiment was replicated four times with batches of microsomes from different livers. After addition of 6% w/v perchloric acid, the samples were centrifuged at 3700 r.p.m. and the supernatant was injected onto a high pressure liquid chromatography system which measured propranolol metabolites by fluorometric detection.

Propofol did not inhibit the formation of any of the three oxidative products of propranolol. However, propofol 28 $\mu\text{mol litre}^{-1}$ inhibited the formation of the R- and S-glucuronides of propranolol by 19% and 22% (95% CI = 16–24% and 17–26%), respectively. Addition of the detergent Brij 58 (0.03% w/v) to the microsomal suspensions resulted in a three-fold increase in glucuronide formation. Inhibition of the formation of the R- and S-glucuronides of propranolol and propofol (28 $\mu\text{mol litre}^{-1}$) in the presence of Brij was 29% and 28% (95% CI 23–35% and 21–35%), respectively.

We conclude that propofol, at these concentrations, inhibits selectively the hepatic microsomal conjugation of propranolol, but not its oxidation. This suggests that propranolol and propofol are substrates of the same glucuronyltransferase isoenzyme.

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EFFECTS OF SUBANAESTHETIC DOSES OF ISOFLURANE AND ENFLURANE ON AUDITORY EVOKED RESPONSES

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Our objective was to investigate the effect of subanaesthetic concentrations of enflurane and isoflurane on the brain stem (BSAER) and long latency auditory evoked responses (LLAER). We were interested in the possible use of AER in automatic control of anaesthesia and required data at smaller concentrations.

Thirty fasting volunteers (mean age 24 yr) had scalp electrodes placed at the vertex and both mastoids. Fifteen subjects were allocated randomly to receive delivered concentrations of 0, 0.31, 0.5, 0.75% isoflurane, placebo or 0, 0.17, 0.42, 0.6% enflurane and 15 received 0, 0.2, 0.31, 0.4% isoflurane or placebo. At each step change in volatile agent concentration, 20 min was allowed for equilibration. The reclining volunteers received the agents through a Hudson mask connected to a Bain circuit (flow > 10 litre min^{-1}), delivering 30% oxygen in air. Basic physiological variables, heart rate, arterial pressure, axillary temperature and oxygen saturation were monitored throughout each 2.5-h experiment. End-tidal concentrations of carbon dioxide were not measured in these conscious volunteers. Control baseline brain stem and long latency recordings were made before introducing the volatile agents or placebo. A probability of $P \leq 0.05$ was considered significant.

The basic physiological variables remained within the normal ranges throughout each experiment. BSAER amplitudes and latencies did not change significantly for either drug compared with placebo, except for wave V latency, which increased significantly at greater inspired concentrations of isoflurane and enflurane.

The N100 latency of the LLAER increased significantly with greater inspired concentrations of isoflurane and enflurane. N100 amplitude differed significantly from placebo. N100 latency

appears to produce a graded response to isoflurane which may be useful in automatic control of anaesthesia.

HALOTHANE ENHANCES THE FORMATION OF INOSITOL (1,4,5) TRIPHOSPHATE MASS IN HUMAN NEUROBLASTOMA CELLS

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As part of a study examining the effects of anaesthetic agents on neuronal signal transduction, we have investigated the effects of halothane on inositol (1,4,5) triphosphate (IP3) mass formation in carbachol-stimulated SH-SY5Y neuroblastoma cells. The muscarinic receptor (M1, M3, M5 subtypes) is an important, well characterized example of a receptor coupled to phosphoinositide turnover and the generation of IP3. IP3 is a second messenger which mobilizes calcium from internal stores and thus has an important role in cellular calcium homeostasis [1].

All experiments were performed at 37 °C in 300 μl of Krebs/HEPES buffer at pH 7.4, and cells were preincubated for 15 min with or without halothane. In order to assess the time course of muscarinic-stimulated IP3 formation, cells were incubated in the presence or absence of carbachol 1 mmol litre^{-1} for 0–300 s. Other cell suspensions were gassed with air or 2% (v/v) halothane in air delivered via a precalibrated Fluotec 3 vaporizer. Reactions were terminated and the IP3 extracted and assayed as described previously [2,3]. Statistical comparisons were made by Student's *t* test.

Carbachol caused biphasic formation of IP3 with a peak (12-fold) at 10 s followed by a plateau, which was significantly ($P < 0.01$) greater (three-fold) than corresponding basal concentrations. Halothane had no effect on basal IP3 concentrations at the start of the experiment but caused a significant ($P < 0.01$) increase at 300 s and significantly ($P < 0.01$) enhanced carbachol-stimulated IP3 formation by two-fold at the peak and three-fold at the plateau (table III).

TABLE III. Effect of halothane on basal and carbachol-stimulated (CS) IP3 formation (pmol/mg protein) in SH-SY5Y cells (mean (SEM), $n = 5$). ** $P < 0.01$ compared with air

	Air	Halothane
Basal 0 s	7.21 (1.11)	11.03 (3.33)
Basal 300 s	7.25 (1.30)	24.33 (2.48)**
CS peak	82.2 (2.89)	167.0 (18.2)**
CS plateau	18.3 (2.38)	56.11 (8.40)**

There are two possible explanations for the stimulatory effect of halothane on IP3 formation. Halothane could activate phospholipase C, the enzyme responsible for IP3 formation, or halothane could inhibit the enzymes responsible for IP3 metabolism. Further studies are required to answer these questions.

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HALOTHANE INHIBITS THE POTASSIUM EVOKED RELEASE OF ACETYLCHOLINE FROM RAT CORTICAL SLICES

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Acetylcholine is an excitatory neurotransmitter which may be

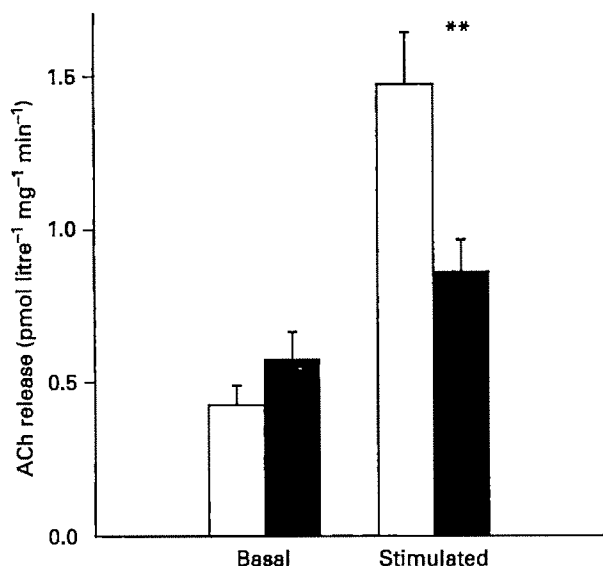


FIG. 1. Basal and K⁺ evoked acetylcholine (ACh) release from rat cortical slices in the presence (■) and absence (□) of 2.4 (rat) MAC of halothane. Data are mean (SEM), $n = 8$. ** $P = 0.01$ compared with control.

important for the maintenance of consciousness. Brain slices were prepared from female Wistar rats [1] and divided into two equal aliquots. One group was incubated in oxygenated Krebs buffer at 37 °C, the other in oxygenated Krebs buffer containing phospholine 65 $\mu\text{mol litre}^{-1}$, an organophosphorus anticholinesterase. Krebs buffer (5 mmol litre⁻¹ of K⁺) and modified buffer (46 mmol litre⁻¹ of K⁺) were equilibrated with carrier gas (5% carbon dioxide in oxygen) or carrier gas containing 2.4 (rat) MAC of halothane. At time 0 min and after 30 min, samples were sedimented and the supernatants assayed for acetylcholine content using a chemoluminescence assay [2].

K⁺ evoked acetylcholine release was decreased significantly in the halothane group compared to the control group (fig. 1). There was no statistical difference in acetylcholine release between halothane and control in the basal group.

These experiments demonstrate that K⁺ evoked acetylcholine release is decreased in rat cortical slices in the presence of 2.4 MAC of halothane. A decrease in acetylcholine release, together with decreased precursor uptake [3], may contribute to the loss of consciousness associated with halothane anaesthesia.

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ANTAGONISM OF HALOTHANE-INDUCED *IN VITRO* CONTRACTURE OF SKELETAL MUSCLE FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE PATIENTS BY DILTIAZEM AND FLUNARIZINE

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Diltiazem has been shown to reduce the halothane-induced *in vitro* contracture response of skeletal muscle from malignant

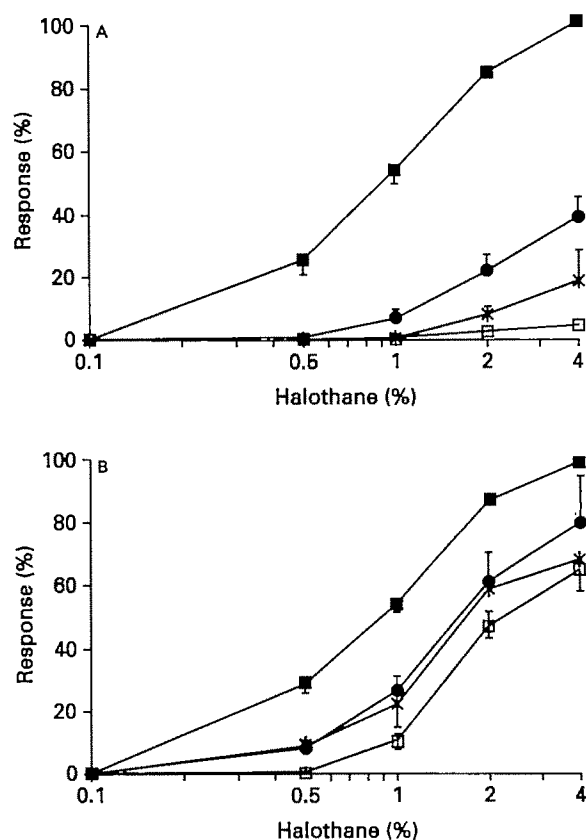


FIG. 2. Contracture responses to incremental additions of halothane in the presence of 1 (●), 10 (*) or 100 (□) $\mu\text{mol litre}^{-1}$ of diltiazem (A) or flunarizine (B). ■ = Control. Values are mean, SEM of at least four experiments.

hyperthermia (MH) susceptible patients [1]. The aim of this study was to characterize this diltiazem antagonism and also, that caused by flunarizine (a chemically unrelated calcium channel antagonist) in order to investigate possible mechanisms for the action of halothane on MH muscle.

Patients attending for diagnostic muscle biopsy were typed according to the European MH Group procedure [2]. Using muscle specimens surplus to diagnostic requirements, the contracture responses to incremental additions of 0.5%, 1%, 2% and 4% halothane were recorded in the presence of 1, 10 or 100 $\mu\text{mol litre}^{-1}$ of either diltiazem or flunarizine. These were compared to those obtained during the diagnostic halothane contracture tests.

The effects of diltiazem and flunarizine are shown in figure 2.

The effect of diltiazem is characteristic of an antagonist, which strongly suggests that, in human MH, halothane is causing an influx of calcium through L type calcium channels.

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MET-ENKEPHALIN INDUCES DEPOLARIZATION OF CULTURED MOLLUSCAN NEURONES IN THE PRESENCE OF HALOTHANE

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Despite increasing evidence for peptides acting as neurotransmitters in the CNS, very little is known about the effects of general anaesthetics on peptidergic systems. We report the effects of the inhalation anaesthetic halothane, on the responses of cultured *Lymnaea stagnalis* neurones to applied met-enkephalin. This opioid peptide was first identified and characterized in

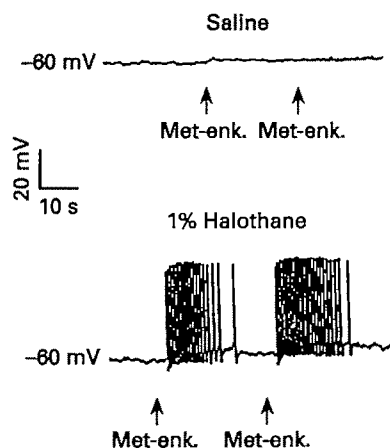


FIG. 3. Effects of met-enkephalin (Met-enk.) $10 \mu\text{mol litre}^{-1}$ on an identified Pedal A cluster neurone.

molluscs by Leung and Stefano [1]. Since then, its presence in other molluscan species (including *Lymnaea*) has been reported.

The Pedal A neurones of the pedal ganglia of *Lymnaea* CNS were cultured in defined medium for 1–2 days. Halothane, prepared at various concentrations in standard HEPES saline, was superfused over individual neurones and the effects recorded.

Halothane 1%, 2% and 4% produced a characteristic initial depolarization and excitation of the Pedal A neurones, followed by hyperpolarization and inhibition. Met-enkephalin was applied at concentrations of $10 \mu\text{mol litre}^{-1}$ and $100 \mu\text{mol litre}^{-1}$ using a puffer pipette attached to a pressure ejection system. In normal HEPES saline, met-enkephalin produced inconsistent effects. However, in the presence of 1% halothane, at the same membrane potential, met-enkephalin produced a large depolarization and increased excitation ($n = 9$), as shown in figure 3. With 2% and 4% halothane, similar effects were observed. Thus in the presence of halothane, met-enkephalin consistently induces depolarization of neuronal membranes.

ACKNOWLEDGEMENTS

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AN INVESTIGATION OF THE LOCAL ANAESTHETIC EFFECTS OF PETHIDINE IN I.V. REGIONAL ANAESTHESIA IN VOLUNTEERS

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Pethidine has been shown to have local anaesthetic actions both *in vitro* [1] and *in vivo* [2]. The aim of our study was to investigate clinically the local anaesthetic properties of pethidine in small concentrations whilst avoiding its systemic effects. This was achieved using an i.v. regional anaesthesia technique [3].

Five healthy, fasted male volunteers were given i.v. regional anaesthesia in a double-blind, randomized fashion with three different test solutions. The solutions were: 0.1% pethidine 40 ml (1 mg ml^{-1}); 0.2% pethidine 40 ml (2 mg ml^{-1}) and 0.5% prilocaine 40 ml as a control. One volunteer was also randomized to receive 0.9% sodium chloride solution 40 ml as a further control. I.v. regional anaesthesia was performed in the non-dominant arm using a standard technique.

I.v. cannulae were inserted into the dorsum of both hands. A pneumatic tourniquet was placed around the upper arm, the arm was exsanguinated, the tourniquet inflated to 250 mm Hg and 40 ml of the test solution injected over 40 s. After the end of injection, sensation and motor function were tested every 1 min on the blindfolded subject. Twenty minutes later, the tourniquet was deflated and testing continued each 1 min until full recovery.

Sensory block was tested at six different sites, representing branches of small peripheral nerves as described previously [3]. Sharp and touch sensations were tested using a 27-gauge short bevelled needle. Cold sensation was assessed using an ethyl chloride spray. Motor power was graded every 5 min as normal (3) to no power (0). The number of sites blocked for sharp, touch and cold sensation was recorded at each time, as was motor power. A graph was plotted for each sensation and motor power.

Sensory impairment occurred with prilocaine and pethidine, but the former had a more profound effect. Sodium chloride 0.9% had little effect. The graph for sharp sensation is shown in figure 4.

Motor power impairment was more marked with prilocaine than with pethidine. All subjects developed wheals with pethidine. With 0.2% pethidine, dizziness occurred in four subjects and one became drowsy.

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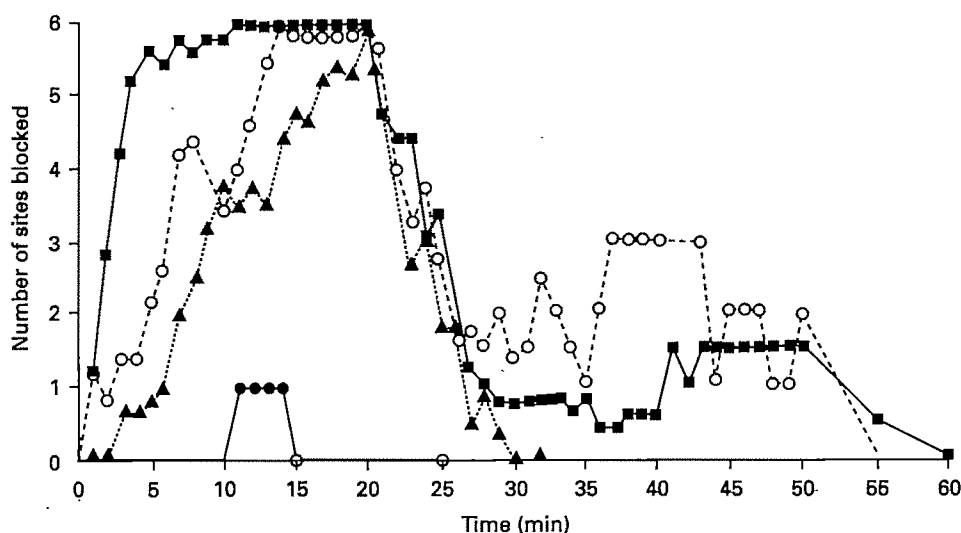


FIG. 4. Number of sites blocked for sharp sensation after administration of 0.5% prilocaine (■), pethidine 1 mg ml^{-1} (▲), pethidine 2 mg ml^{-1} (○) and saline (●).

FOLLOW-UP OF DAY-CASE PATIENTS IN A DISTRICT GENERAL HOSPITAL

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Day-case surgery is becoming increasingly popular because of its benefits to the patients and provider of the service [1]. We investigated if the quality of care for our day-case patients was being maintained.

A total of 200 consecutive day-case patients were sent a brief postal questionnaire less than 3 weeks after discharge. The questionnaire consisted of 10 questions and a linear analogue scale concerning standard of care. There were 166 of 200 (83%) respondents. Three had their surgery cancelled and therefore 163 results were analysed.

Only 84% of patients had general anaesthesia. Ninety percent of patients said they had received instructions before operation, which are routinely sent out with the appointment. All those that received them understood the instructions, although it was found that 77% of patients thought that GP prescribed medications should not be taken on the day of operation. This could cause problems if antihypertensives or other essential medications were omitted.

It is considered mandatory in our department to allow at least 2 h after recovery from general anaesthesia before discharge. It was interesting to find that 36% of patients were discharged home 0–2 h after recovery, 42% 2–4 h and 22% more than 4 h.

Private car (90%) was the most popular method of getting home, followed by taxi (9%), with 1% using the bus or walking. Of those using private cars, 97% did not drive. Of the five patients who drove, four had had local anaesthesia. The single patient who drove himself did so within 2 h of recovery: 94% of all patients were accompanied home.

Only 39% of patients were given written instructions on discharge and all were understood. It was therefore not surprising to find that 57% of the general anaesthesia group and 92% of the local anaesthesia group used machinery (cooker or kettle) in the first 24 h. Only 8% of patients worked the day after discharge.

After operation, 14% of patients suffered more pain than expected and 18% complained of sore throat (59% of these were intubated for non-ENT surgery). Muscle pain was noted in 34 patients (21%) after general anaesthesia. Of these, 21 had received suxamethonium without pretreatment. Two patients complained specifically of pain at the site of injection of analgesia. Both had received i.m. diclofenac which is known to cause prolonged pain at its injection site [2].

A linear analogue scale (0–10) of general satisfaction showed a mean result of 8.56 (range 2–10).

In conclusion, it appears from this preliminary study that patients are satisfied with day care. However, there is a definite need to improve the provision of instructions and decrease postoperative morbidity.

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ACTIVE SOLUTE TRANSPORT ANTAGONIZES ALVEOLAR FLOODING ACROSS THE FULLY WETTED *IN VIVO* RABBIT ALVEOLAR EPITHELIUM

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Hills has proposed that the alveolar epithelium prevents alveolar flooding by a mechanism of water repellency rather than the widely assumed action of Starling forces across a membrane with a reflection coefficient for plasma proteins close to unity [1]. An associated hypothesis is that wetting of the reputedly normally dry alveolar epithelium leads to alveolar flooding as a result of unopposed pulmonary capillary hydrostatic pressure [1]. We have tested this hypothesis in adult rabbits.

Animals (weights 2.9–3.5 kg) were anaesthetized using halothane and Hypnorm (fentanyl-fluanisone), paralysed with alcu-

ronium and tracheostomized supine. The left main bronchus was isolated with a balloon tipped catheter, through which the lung was filled with a solution of glucose 10 mmol litre⁻¹ to which had been added sufficient sodium chloride to make the solution isotonic with plasma. The catheter was then connected to a reservoir of the solution maintained at a constant pressure of 3 cm H₂O relative to the manubrium. In eight animals, amiloride 1 mmol litre⁻¹ and phloridzin 1 mmol litre⁻¹ were included in the solution to block apical sodium channels and sodium–glucose co-transport, respectively [2]; six animals acted as controls. The right lung was ventilated with oxygen using a positive end-expiratory pressure of 3 cm H₂O and a mean airway pressure 6 cm H₂O to maintain normocapnia. After 200 min of equilibration, the bronchial liquid flow was measured over 160 min.

In control animals, bronchial liquid flow was *into* the alveoli at mean 21.9 (SEM 4.5) µl min⁻¹. In animals given amiloride and phloridzin in the instillate, bronchial liquid flow was *out of* the alveoli at 4.1 (1.5) µl min⁻¹. Each reading differed significantly from zero at the 5% level on *t* testing.

These findings support Hills' hypothesis, but only in so far as it relates to an epithelium in which active sodium transport is inhibited. The observation that wetted "inhibited" alveoli flood at a pressure of 3 cm H₂O suggests that the reflection coefficient for the alveolar epithelium is small. The data also suggest that active solute transport may have an important role in preventing alveolar flooding. The question of whether or not active transport is compromised in respiratory distress syndromes needs to be addressed [3].

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PREDICTING DIFFICULT INTUBATION: A NEW LOOK AT THE UNDERLYING STATISTICAL THEORY

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A strategy outlined previously [1] has been applied to long-standing problems in discriminant function analysis [2]. Experiments with computer simulations indicated that the population variables are best estimated using means and SD as in the raw data, but with *n* increased to 5000. This eliminated misleading results given by commercial software.

Similarly, the confidence limits of *D* (the Mahalanobis gap) were found from experimentally generated *D* values. Plotting SD of *D* against $1/\sqrt{n}$ gave a straight line through the origin (fig. 5), deviating only in small samples. Thus the SD of *D* is found simply from the equation:

$$SD = g/\sqrt{n}$$

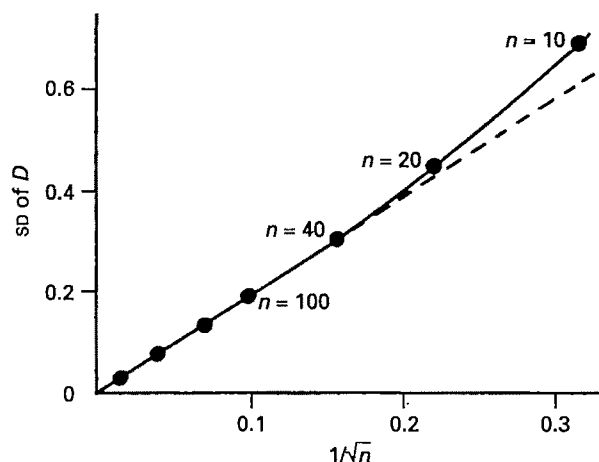


FIG. 5. SD of experimentally determined *D* values varies with *n*, when mean *D* = 2.483.

where g is constant for a particular D , but varies with different D values. The relation of g to D was found to be $g = 0.2999D + 1.143$ (when D is between 1.6 and 2.5). Thus despite the complexity of the underlying distribution of D , its confidence limits are easier to find than those for a routine t test. The method is exact for large samples, but if $n < 100$ then the result should be checked against computer simulations. If 95% of the experimental D values lie between limits predicted from the equations above, then that is correct. But if, on the other hand, the D values are skewed to the right, they provide a more realistic description. We conclude that if orthodox mathematics fails then the advance of statistical theory may be achieved by experiment—the strategy of science.

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A NEW TUBE FOR GRADE 3 DIFFICULT INTUBATIONS

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A new Portex tracheal tube was compared with the Oxford tube in performing simulated grade 3 difficult intubations. The Portex tube was modified so that the bevel faced backwards as in the Oxford tube, to avoid the problems that arise when an introducer is needed [1]. The intubations were performed by the authors (M.J. and M.W.), who had approximately 2 years' experience. A gum elastic introducer was used with both tubes. The time taken and the number of attempts needed to achieve success were recorded, as were changes in arterial pressure and heart rate, and incidence of sore throat. Both tracheal tubes were easy to insert and there were no significant differences between them. The first attempt was successful in 65% of cases.

We conclude that the new tube is suitable for both routine and grade 3 cases with advantages of safety, cost and convenience. An unexpected but important finding was a clear learning effect, despite both anaesthetists being familiar with the techniques at the outset. This demonstrates the importance of practising blind intubation as others have advocated [2].

A least squares learning curve (fig. 6) was fitted to the pooled data, using a standard method [3]:

$$Y_n = Y_0 \cdot e^{-k \cdot n} + Y_\infty$$

where Y_n = time taken after n intubations; Y_∞ = asymptote,

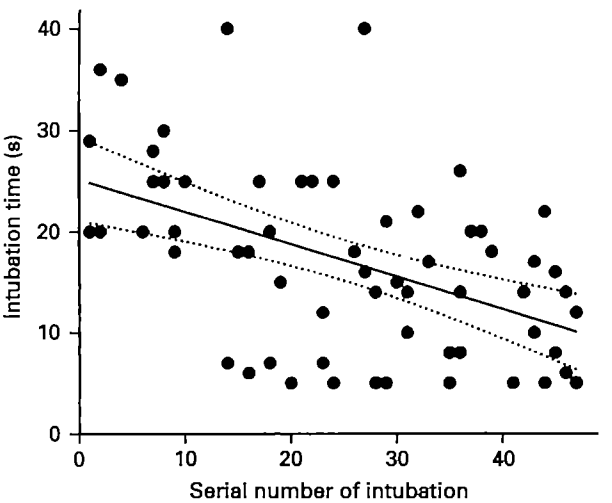


FIG. 6. Time taken to complete simulated grade 3 difficult intubations. Pooled data ($n = 62$) for all intubations (both tubes and both anaesthetists).

“expert time” (9.9); Y_0 = initial time, less asymptote (19.0); k = learning constant (0.047) (best fit values).

In a small sample these estimates are of course approximate, nevertheless they are the most likely values for this data set. A learning constant of 0.047 corresponds to a half-life of 15, that is, the number of intubations needed to halve the distance between the “novice time” ($Y_0 + Y_\infty$) and the expert time (Y_∞). If that distance is 20, then three half-lives or 45 intubations are needed to get within 2.5 of the expert time (fig. 6). We conclude that trainees should aim to perform at least 30 simulated difficult intubations before handling a high-risk case.

ACKNOWLEDGEMENT

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INTERACTION OF VECURONIUM AND PANCURONIUM WITH CARDIAC M2 MUSCARINIC RECEPTORS

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Many non-depolarizing neuromuscular blocking agents have cardiovascular effects. Clinically, pancuronium produces tachycardia and increases in arterial pressure. In contrast, vecuronium has been reported to have minimal effects on the cardiovascular system and has even been associated with a few cases of bradyarrhythmias [1]. We have examined the binding of both vecuronium and pancuronium to M2 muscarinic receptors on a rat heart membrane preparation using [^{3} H]-N-methyl scopolamine (NMS) as a ligand.

All displacement assays were performed in 1-ml volumes of HEPES 20 mmol litre⁻¹, Mg²⁺ 1 mmol litre⁻¹ buffer, pH 7.4, at 37 °C for 60 min. The affinity (K_{50}) of vecuronium, pancuronium and the M2 selective antagonist methoctramine were determined by the displacement of NMS (approximately 0.4 nmol litre⁻¹). Bound and free ligand were separated by vacuum filtration.

The binding of NMS to rat heart membranes was dose-dependent and saturable with a K_d value of 0.26 nmol litre⁻¹ and B_{max} of 104 fmol/mg protein, respectively. As can be seen in table IV, the M2 selective antagonist methoctramine displayed a nanomolar affinity and slope factor of unity, consistent with the presence of a homogenous population of M2 muscarinic receptors. More importantly, both vecuronium and pancuronium displayed affinity values that lay within the serum concentrations seen during clinical anaesthesia.

In conclusion, we have demonstrated an interaction of both vecuronium and pancuronium with cardiac M2 muscarinic receptors which may be responsible for cardiac effects produced by these drugs. The nature of this interaction, i.e. agonist, antagonist, allosteric [3], is currently under investigation.

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TABLE IV. Mean (SEM) affinity values (K_{50}) for methoctramine, vecuronium and pancuronium. **P < 0.01 compared with vecuronium

Drug	K_{50} (nmol litre ⁻¹)	Slope factor
Methoctramine	6.5 (0.5)	1.08 (0.05)
Vecuronium	549.9 (37.0)	0.60 (0.01)
Pancuronium	192.2 (21.6)**	0.49 (0.02)**

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COMPARISON OF THE HAEMODYNAMIC EFFECTS OF ROCURONIUM AND VECURONIUM

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INTUBATING CONDITIONS AND NEUROMUSCULAR EFFECTS AFTER MIVACURIUM WITH OR WITHOUT PRIOR SUXAMETHONIUM

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Mivacurium is a new non-depolarizing neuromuscular blocking agent metabolized by plasma cholinesterase and has a relatively short duration of action [1]. The present study was undertaken to assess the intubating conditions after its use and to study its neuromuscular effects with and without prior administration of suxamethonium.

Sixty healthy adults undergoing elective surgery were included in the study. Anaesthesia was induced with fentanyl 1-3 µg kg⁻¹ and thiopentone 3-5 mg kg⁻¹ and maintained with nitrous oxide in oxygen until intubation after which 0.5 to 1.0% halothane was added to the inspired gas mixture. The ulnar nerve was stimulated at the wrist using a train-of-four mode of stimulation at 2 Hz every 12 s and recording the force of contraction of the adductor pollicis muscle. The first 40 patients were randomized to two groups of 20 each to have the trachea intubated at 1 min after suxamethonium 1 mg kg⁻¹ or at 2 min after mivacurium 0.15 mg kg⁻¹. Intubating conditions were assessed according to a previously described scheme [2]. Because of uniformly poor intubating conditions with mivacurium, the study was restarted using 0.2 mg kg⁻¹. The last 20 patients also received mivacurium 0.2 mg kg⁻¹ but were intubated at 2.5 min. The same dose of mivacurium was given to patients receiving suxamethonium after recovery to 90% of control. The times to onset of block and spontaneous recovery were recorded in all patients. The data were subjected to analysis of variance and chi-square tests.

The intubating conditions were excellent or good in all patients after suxamethonium and in 13 of 20 and 16 of 20 patients, 2 and 2.5 min after mivacurium. The differences between mivacurium and suxamethonium were significant ($P < 0.05$) but not between the two mivacurium groups. Data are shown in table V. The onset of action was slower and the duration of action longer in the mivacurium groups compared with suxamethonium ($P < 0.05$). Prior suxamethonium administration had no significant effect on the actions of mivacurium.

TABLE V. Mean (SD) neuromuscular effects of suxamethonium (Sux.) and mivacurium. RI = Recovery index. There were significant differences ($P < 0.05$) between Sux. and mivacurium groups

	Sux. group	Mivacurium group	
		Without prior Sux.	With prior Sux.
<i>n</i>	20	40	20
Onset (s)	48.6 (4.8)	96.7 (22.7)	86.9 (22.2)
Recovery of T1 to			
25% (min)	10.1 (3.0)	17.9 (4.5)	18.4 (5.9)
90% (min)	13.5 (4.0)	25.8 (7.4)	26.5 (8.7)
RI (min)	2.4 (1.2)	5.5 (1.8)	5.7 (2.0)
Time to TOF = 0.7 (min)		27.0 (6.0)	30.0 (8.4)

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PHARMACOKINETICS OF THE ISOMERS OF MIVACURIUM CHLORIDE IN HEALTHY PATIENTS AND IN RENAL FAILURE

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A solution of mivacurium chloride contains three non-interchangeable stereoisomers: *trans-trans* (57%), *cis-trans* (37%) and *cis-cis* (6%). We have presented previously the pharmacokinetics of mivacurium in healthy patients following a 10-min infusion, and shown a correlation between plasma cholinesterase activity and clearance of the *trans-trans* and *cis-trans*, but not the *cis-cis* isomer [1]. We determined the pharmacokinetics of mivacurium during a prolonged infusion, at a variable rate, in healthy patients and in those with end-stage renal failure.

Four healthy and five renal failure patients (creatinine clearance < 20 ml min⁻¹), undergoing surgical procedures of at least 1 h duration, were studied. Plasma cholinesterase activity was measured before operation. After premedication with oral diazepam 10 mg, anaesthesia was induced with midazolam 0.05 mg kg⁻¹, fentanyl 1 µg kg⁻¹ and thiopentone 3-5 mg kg⁻¹, and maintained with 70% nitrous oxide in oxygen with additional doses of fentanyl and thiopentone as appropriate. An i.v. cannula for infusion of mivacurium was inserted in one limb and a separate 14-gauge cannula for blood sampling was placed in the arm used to monitor the electromyographic response to the neuromuscular blocking agent throughout anaesthesia. After a baseline blood sample had been obtained, mivacurium was infused using an Ohmeda 9000 pump at a rate of 15 µg kg⁻¹ min⁻¹ for 10 min, 7.5 µg kg⁻¹ min⁻¹ for the next 10 min and then at a rate adjusted to maintain T1/T0 at 5%. Blood samples (7 ml) were obtained at 1, 2, 5, 10, 11, 12, 15, 20, 30, 45 and 60 min and at every 30 min for the duration of the infusion, and then at 0, 1, 2, 5, 10, 15, 30, 45, 60, 120, 180, 240 and 300 min after termination of the infusion. Blood was placed immediately into lithium-heparin bottles containing phospholine iodide 1 mg to inhibit plasma cholinesterase. Plasma was separated and frozen until analysis. Mivacurium was extracted from plasma using a solid-phase technique. The isomers were separated using HPLC and assayed fluorimetrically [1].

We used three standard methods to estimate the clearance of each isomer: (1) the standard formula, dose/AUC; (2) clearance at steady state; and (3) by fitting a compartmental model.

Patients were aged 24-64 yr and weighed 40-75 kg. Plasma cholinesterase concentrations were 373-1179 iu litre⁻¹. Creatinine clearance in the healthy group ranged from 66 to 104 ml min⁻¹ and in the renal failure group from 4 to 11 ml min⁻¹. Clearance estimated by the three methods is shown in table VI.

Clearance of the *cis-trans* and *trans-trans* isomers correlated significantly with plasma cholinesterase activity using all three methods ($r = 0.70$, $P < 0.05$). There was no correlation between cholinesterase activity and clearance of the *cis-cis* isomer estimated

TABLE VI. Clearance (Cl) (ml kg⁻¹ min⁻¹) of each isomer estimated by the three methods and model-derived values of Vd (ml kg⁻¹) and T_{1/2} (min) for all patients. Values are mean (SD)

	Cis-cis	Cis-trans	Trans-trans
Cl (Dose/AUC)	3.2 (1.1)	90 (50)	50 (26)
Cl (C _p ^{ss})	4.3 (1.6)	109 (90)	58 (39)
Model			
Cl	3.7 (1.1)	93 (49)	51 (25)
Vd	175 (42)	382 (205)	267 (168)
T _{1/2}	34.7 (9.5)	2.9 (1.1)	3.6 (1.4)

by the AUC and model fitting methods, but a significant correlation with clearance of the *cis-cis* isomer estimated at steady state ($r = 0.88$, $P < 0.001$). There was no correlation between creatinine clearance and clearance of any of the isomers.

Estimation of volume of distribution and half-life for the more rapidly cleared isomers is subject to difficulties.

ACKNOWLEDGEMENT

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EFFECT OF FOUR ANAESTHETIC INDUCTION AGENTS ON PHA-INDUCED T-LYMPHOCYTE TRANSFORMATION

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Anaesthetic induction agents may cause transient suppression of the human immune system. Thiopentone has been shown to inhibit several factors [1] but there is little information on methohexitone and etomidate. Preliminary data concerning propofol suggest no depression [2]. We have investigated the effect of four i.v. anaesthetics and solubilizing agent on T-lymphocyte transformations *in vitro*.

The four agents and 10% Intralipid were studied at two concentrations: thiopentone 40 and 400 $\mu\text{g ml}^{-1}$; methohexitone and etomidate 10 and 100 $\mu\text{g ml}^{-1}$; propofol 6 and 60 $\mu\text{g ml}^{-1}$. Lymphocytes were separated from blood samples obtained from healthy blood donors using a standard laboratory technique. These were incubated in microtitre wells for 48 h at 37 °C in 5% carbon dioxide in air, with phytohaemagglutinin (PHA), a T-cell specific mitogen, at the following levels: 0, 0.25, 0.5, 1, 2 and 4 $\mu\text{g ml}^{-1}$. Tritiated thymidine 50 μl was added and the cells incubated for a further 8 h under the same conditions. Harvesting was performed and scintillation fluid added allowing counts to be made. The average of four wells was noted. Results were analysed using Friedman's analysis of variance of ranks and the Wilcoxon signed rank test.

Results are shown in table VII. Thiopentone, at the clinical concentration, showed a decrease in counts which was just outside the level of statistical significance ($0.05 < P < 0.1$). The greater concentration produced total inhibition of cell transformation. Methohexitone and etomidate showed significantly decreased counts at both concentrations ($P < 0.05$ and $P < 0.01$). Propofol was associated with a small increase in counts in the absence of PHA. The reduction in counts at greater concentrations of PHA was not significant with either concentration of propofol. Intralipid 10% showed no significant decrease in counts at any concentration.

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THE NATURE OF FREE RADICALS WITH WHICH 2,6-DIISOPROPYLPHENOL INTERACTS

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2,6-Diisopropylphenol (propofol) is structurally similar to butylated hydroxytoluene, an important free radical scavenger. Recent work suggests that propofol may also have free radical scavenging properties [1]. The aim of this study was to determine with which free radicals propofol may act as a scavenger.

We determined the effect of propofol on the decrease in oxygen tension during different free radical generating reactions and investigated structural changes to propofol indicating oxidation. Three free radical generating systems were used: (1) The Fenton reaction which produces hydroxyl and superoxide radicals; (2) xanthine and xanthine oxidase which also produce superoxide radicals; and (3) riboflavin photolysis which produces superoxide, organic radicals and singlet oxygen [2].

Each system was placed in the reaction chamber of an oxygen analyser using a Clark electrode and the rate of change of oxygen tension recorded as a measure of free radical production. This was compared with the rate of change of oxygen tension in the presence of propofol in alcohol, propofol in Intralipid (Diprivan), and butylated hydroxytoluene in alcohol. After reaction, the residual solution was mixed with hexane to extract the organic components. This extract was then analysed by HPLC. Second, we looked at the effect of propofol on the reduction of nitroblue tetrazolium by free radicals to blue coloured formazan. This reaction was measured spectroscopically, and then repeated with the addition of superoxide dismutase to remove superoxide free radicals.

Oxygen uptake was altered conclusively only in the riboflavin/photolysis free radical generating system where propofol slowed oxygen consumption at concentrations greater than 10 $\mu\text{g ml}^{-1}$. HPLC analysis of the products from the riboflavin generated reaction suggested breakdown of propofol not seen in the Fenton or xanthine reactions. By adding superoxide dismutase to the nitroblue tetrazolium reaction, we showed that the action of propofol as a free radical scavenger occurred independent of the presence of superoxide.

Propofol is a scavenger of organic radicals but not of superoxide radicals.

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COMPARISON OF CIRCULATORY EFFECTS OF HIGH FREQUENCY VENTILATION AND HIGH FREQUENCY OSCILLATION TO CONVENTIONAL MECHANICAL VENTILATION WITH POSITIVE END-EXPIRATORY PRESSURE IN THE SURFACTANT DEFICIENT RABBIT

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Positive end-expiratory pressure (PEEP) when added to improve oxygenation in the surfactant depleted lung, may reduce cardiac

TABLE VII. Mean (SEM) counts per min after incubation with PHA 4 $\mu\text{g ml}^{-1}$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Drug	Control	Clinical concn	10 × clinical concn
Thiopentone	103117 (15242)	91950 (11892)	297 (50)***
Methohexitone	103099 (9170)	108334 (13613)*	60621 (3515)**
Etomidate	117655 (16258)	98402 (15065)*	1232 (519)***
Propofol	138107 (23822)	135340 (22619)	120240 (18733)
Intralipid	107908 (11229)	106959 (11437)	97929 (8407)

TABLE VIII. Mean (SEM) aortic blood flow (ml min^{-1}) at 0, 5 and 10 $\text{cm H}_2\text{O}$ PEEP before and after lavage. Significance differences: * $P < 0.05$, ** $P < 0.01$ compared with 0 PEEP within groups; †† $P < 0.01$ between groups with same PEEP and lung status

Ventilatory mode	PEEP ($\text{cm H}_2\text{O}$)		
	0	5	10
CMV			
Normal lung	377 (26)	224 (31)**	163 (31)**
Lavaged	327 (31)	215 (25)*	89 (17)**
HFV			
Normal lung	337 (32)	226 (22)**	144 (32)**
Lavaged	190 (31)††	230 (43)	146 (24)
HFO			
Normal lung	341 (56)	258 (55)	175 (39)**
Lavaged	201 (29)††	198 (23)	149 (25)

output and thus oxygen transport [1]. High frequency ventilation (HFV) with passive expiration and high frequency oscillation (HFO) with active expiration theoretically produce lower airway pressures compared with conventional mechanical ventilation (CMV) for the same PEEP level and may prevent circulatory depression.

Six New Zealand white rabbits were anaesthetized and measurements of airway pressures, arterial pressure, central venous pressure, heart rate, blood-gases and pH, and aortic blood flow were made as described previously [1]. A valveless jet driven ventilator, a prototype of the SLE 2000, was used to ventilate at 0.5 and 10 $\text{cm H}_2\text{O}$ PEEP in two modes of ventilation: CMV 30 b.p.m. I:E 1:2 and HFV 300 b.p.m. I:E 1:2, and a prototype jet driven oscillating ventilator was used to provide HFO 300 b.p.m. I:E 1:1. The rabbits' lungs were lavaged with saline to simulate neonatal respiratory distress syndrome [2] until PaO_2 was less than 10 kPa with FiO_2 1.0. Increased airway pressure was provided during CMV to maintain normocapnia. The three ventilatory modes at the three PEEP values were repeated at random and measurements made at approximately 30 min after a change in ventilation, provided that serial blood-gases were stable. Data were analysed using repeated measures analysis of variance and $P < 0.05$ was accepted as significant.

Aortic blood flow decreased significantly as PEEP was increased in normal rabbits' lungs (table VIII) and this was associated with an increase in central venous pressure and a decrease in mean arterial pressure during all modes of ventilation. After lavage, blood flow with CMV followed the same pattern but at greater PEEP values with large peak airway pressures, blood flow was reduced compared with normal lung values. The use of HFV and HFO with PEEP maintained aortic blood flow in the lung lavage model but was not associated with significant changes in oxygen tension. At zero PEEP, PaO_2 was maximal during HFO, but this did not reach statistical significance. The peak airway pressures were significantly smaller during HFV and HFO compared with CMV. However, they were similar during HFV and HFO.

Circulatory depression was minimized in the rabbit lung lavage model of respiratory distress syndrome by the use of large frequencies of ventilation, which means that the oxygen supply may be better in these modes of ventilation.

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COMPARISON OF PREDICTED AND MEASURED CREATININE CLEARANCE IN POSTOPERATIVE CARDIAC SURGERY PATIENTS

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Previous studies have validated methods to predict creatinine clearance from a single plasma creatinine concentration in patients

with both normal and abnormal renal function [1]. However, it has been emphasized that creatinine concentration has to be stable for at least 4 days to allow valid prediction [2]. This study was designed to investigate if prediction of creatinine clearance from the plasma creatinine concentration is valid in the postoperative period in cardiac surgery patients.

The study was approved by the local Ethics Committee and informed consent was obtained from each patient. All patients were undergoing cardiac surgery. The investigation was conducted over a 2-month period. Patient days before but not including periods of haemodialysis or haemofiltration were studied. Sex, age and body weight of patients were recorded. Blood samples and 4-h urine collections were obtained daily.

Plasma creatinine concentration was determined by a modified Jaffe method using an alkaline picrate reaction. Creatinine clearance was predicted from plasma creatinine concentration utilizing the nomogram described by Siersbaek-Nielsen and colleagues [3]. Statistical analysis by paired t test was applied for comparison of parametric data and regression analysis applied to obtain correlation coefficients.

A total of 93 patient days were studied up to and including the fifth postoperative day. There was a significant correlation between the predicted and measured creatinine clearance on each of the postoperative days (day 1: $n = 33$, $r = 0.92$, $P < 0.001$; day 2: $n = 22$, $r = 0.84$, $P < 0.001$; day 3: $n = 20$, $r = 0.90$, $P < 0.001$; day 4: $n = 11$, $r = 0.95$, $P < 0.001$; day 5: $n = 7$, $r = 0.76$, $P < 0.047$). The correlation was maintained even if the patients required inotrope or vasoconstrictor therapy, were receiving parenteral nutrition or had changing renal function ($r = 0.76-0.95$, $P < 0.05$).

Predicted creatinine clearance is as reliable as measured creatinine clearance in cardiac surgery patients up to the fifth day after operation.

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PSYCHOMOTOR EFFECTS OF 3-15% NITROUS OXIDE INHALATION

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Nitrous oxide is a useful reference compound for assessing the sedative effects of other drugs. It is non-toxic with short-term use, has a short duration of action and steady state may be obtained rapidly. The threshold concentration required for detectable psychomotor effects is still uncertain. Impairment was seen when 0.05% nitrous oxide was inhaled for 4 h [1]. In contrast, 5% had no effect and 8% had only a minor effect on psychomotor performance [2]. The present study was designed to assess the effects on the CNS of inhalation of 3-15% nitrous oxide using a variety of psychomotor and cognitive tests.

Twelve volunteers participated in a randomized, double-blind, six-period crossover study, inhaling 0% (placebo), 3%, 5%, 7%, 10% and 15% nitrous oxide in oxygen. The gas was delivered to a tight fitting mask using a non-rebreathing circuit with a 4 litre reservoir. Gas concentration was measured using the Bruel and Kjaer multigas monitor. Oxygen was breathed for 15 min, then the test gas for 45 min and finally oxygen for a further 15 min. Psychomotor and cognitive tests were administered using a BBC Master microcomputer [3]. A short battery of tests (symbol-digit substitution (SDST), digit-symbol substitution (DSST) and visual analogue scores (VAS) (to assess sedation and mood)) were completed at intervals throughout this period. Tests used during the test gas inhalation included continuous attention task (CAT), choice reaction time (CRT), sentence verification task (SEVT), Buschke selective reminding (BSRT) and masked pattern block recognition test (MPBR).

Compared with placebo, 15% nitrous oxide impaired performance of all tests except SEVT and produced significant changes in VAS. In addition, 7% nitrous oxide affected CAT and

concentrations $\geq 3\%$ affected BSRT. Performance of the short battery of tests was impaired within 5 min of starting to breathe the test gas, recovery was complete 10 min after finishing it.

We conclude that inhalation of 15% nitrous oxide causes global impairment of performance. Lower concentrations have less effect, although there was evidence of memory impairment at concentrations as small as 3%.

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OXYGEN DILUTION IN "TRUNK" VENTILATION OF CIRCLE SYSTEMS (PART 1)

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Circle and to-and-fro anaesthetic systems can be conveniently ventilated by attaching a pressure-cycled ventilator operating as a flow generator. This has advantages [1] but a drawback is that under some circumstances driving gas can enter the anaesthetic system diluting the anaesthetic gases. This could result in awareness in paralysed patients. One cause of such a dilution is an inadequate volume of the reservoir tube (trunk) connecting the ventilator to the anaesthetic system. The volume required in the trunk has been investigated by several workers using model systems [2,3].

Twenty-four subjects were anaesthetized using propofol and ventilation was controlled using nitrous oxide and isoflurane via a laryngeal mask. Patients were attached to a Scania circle system and tidal volumes were measured by a Wright's respirometer connected between ventilator and trunk and adjusted to maintain P_{ETCO_2} between 4 and 4.5 kPa. The system was made up of plastic corrugated disposable tubing (internal diameter 22 mm) (Intersurgical). Oxygen concentration within the anaesthetic system was measured using a calibrated Datex Capnomac sampling at the laryngeal mask. The trunk consisted of a series of detachable segments each having a volume of 50 ml. At each of the fresh gas flow rates (FGF) studied (6, 3 and 1.5 litre $^{-1}$), the length of the trunk was reduced by 50-ml decrements until an increase in oxygen concentration showed that the gas sampled at the laryngeal mask had been diluted by ventilator driving gas. Analysis of the data failed to show any influence of FGF on the trunk volume necessary to prevent this dilution. Therefore, the three sets of data points were averaged and are shown in figure 7.

A linear relation between trunk and tidal volume was found but the minimum trunk volume to prevent dilution was 70 ml less than the tidal volume. It may be that this depended upon the

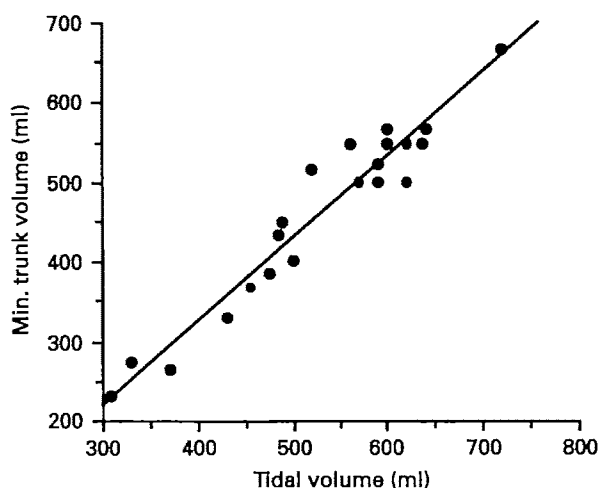


FIG. 7. Relation between trunk and tidal volume ($r = 0.931$).

arrangement of components in the circle system used, a portion of which may function as additional reservoir volume.

For the FGF we studied, the common recommendation of 1.5 litre [3] for the volume of the trunk is generous, but this may not be correct for smaller fresh gas flow rates. The use of monitoring allows this volume to be adjusted appropriately.

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CHANGE IN CHEST WALL DIMENSIONS ON INDUCTION OF ANAESTHESIA. A REAPPRAISAL

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In supine humans, induction of anaesthesia with or without neuromuscular block is associated with a reduction in FRC. Possible explanations for this are an increase in gas trapping, an increase in central blood volume or a reduction in the volume contained within the chest wall [1]. Previous studies do not support the first two theories. Studies of the third possibility, by measuring chest wall dimensions at induction of anaesthesia, have produced conflicting results. Those which inferred volume from linear measurements failed to demonstrate any change, whilst those which used three-dimensional imaging techniques showed a decrease in the volume contained within the chest wall. This could be because earlier studies did not take into consideration possible changes in volume caused by spinal movements, which may take place as muscle tone is lost with induction of anaesthesia. We assessed the changes that could occur in chest wall dimensions after i.v. induction of anaesthesia, when spinal movement was prevented.

Twenty patients gave informed consent for the study, which was approved by the Hospital Ethics Committee. Premedication comprised temazepam 20 mg orally. On arrival in the anaesthetic room, a "Vac Pac" (Howmedica) was placed beneath the patient and the air evacuated to provide a firm conforming support for the thoraco-lumbar spine. Magnetometer coils were placed to measure the anteroposterior (AP) diameter of the chest at the midpoint of the sternum and the AP diameter of the abdomen at the umbilicus. Signals from the coils were recorded on a chart recorder before, during and after induction of anaesthesia with i.v. methohexitone 0.8-1.5 mg kg $^{-1}$. The difference in AP diameter of the chest (ΔRC) and the abdomen (ΔAb) between awake and anaesthetized states was calculated. ΔRC was plotted against ΔAb for each patient, as described by Konno and Mead [2]. In normal supine subjects the relative motion of rib cage and abdomen, in the absence of any volume change, is represented on such a graph by the "isovolume line" which has a slope of -16° to -36° [3]. Movement away from the isovolume line indicates a change in chest wall volume.

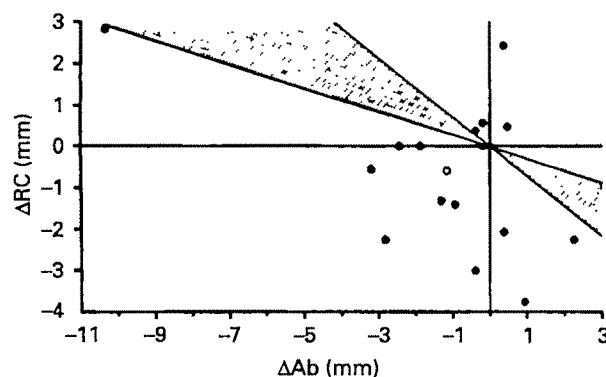


FIG. 8. Difference in anteroposterior diameter of chest (ΔRC) and abdomen (ΔAb) between awake and anaesthetized states. ● = Individual patients; ○ = Mean. Shaded area = isovolume line.

Complete results were available from 17 subjects. The AP diameter of the chest decreased in eight, was unchanged in four and increased in five patients. The AP diameter of the abdomen decreased in 10, was unchanged in two and increased in five patients. Relative to the isovolume line there was a decrease in chest wall volume in 11, no change in two and an increase in four patients (fig. 8).

We conclude that in the majority of patients, after i.v. induction of anaesthesia, there is a decrease in the volume contained within the chest wall. This may explain the change in FRC associated with induction of anaesthesia.

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VARIATION IN THE ARTERIAL TO END-TIDAL P_{CO_2} DIFFERENCE DURING ONE-LUNG THORACIC ANAESTHESIA

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Because of the inevitable intrapulmonary shunt that occurs during one-lung anaesthesia, oxygenation is the primary concern. However, little is known about the value of end-tidal P_{CO_2} (PE_{CO_2}) in reflecting changes in arterial P_{CO_2} (Pa_{CO_2}) in this situation [1]. The present study was designed to estimate the arterial to end-tidal P_{CO_2} difference ($Pa_{CO_2} - PE_{CO_2}$) in the lateral position and to evaluate the practice of correcting the estimate by an initial measurement of ($Pa_{CO_2} - PE_{CO_2}$) during one-lung ventilation.

We studied 22 patients undergoing pulmonary resection in the lateral position. Anaesthesia was maintained using 1.3% isoflurane in oxygen and one-lung ventilation was achieved using a plastic double-lumen endobronchial tube. Ventilation was standardized to a tidal volume of 10 ml kg^{-1} at a rate of 10 b.p.m. Paired measurements of PE_{CO_2} from a capnometer (Datex) and Pa_{CO_2} from arterial blood were obtained. Initial measurements were taken on two-lung ventilation in the lateral position and after transition to one-lung ventilation. Thereafter, repeated pairs of measurements were carried out at 10-min intervals.

($Pa_{CO_2} - PE_{CO_2}$) on two-lung ventilation was compared to the initial measurement on one-lung ventilation using the Student's paired *t* test. On one-lung ventilation for each patient, after computing ($Pa_{CO_2} - PE_{CO_2}$) at each sample point we subtracted the initial ($Pa_{CO_2} - PE_{CO_2}$) from subsequent values to obtain a corrected difference. We plotted the mean (SD) ($Pa_{CO_2} - PE_{CO_2}$) as well as the mean corrected difference for each patient. Net and corrected differences were analysed using ANOVA.

($Pa_{CO_2} - PE_{CO_2}$) during two- and one-lung ventilation were similar (mean (SD) 1.3 (0.6) vs 1.2 (0.7) kPa, respectively). Mean values of ($Pa_{CO_2} - PE_{CO_2}$) varied from 0.2 to 2.5 kPa, while maximum values ranged from 0.3 to 2.8 kPa. Mean (SD) of 133 pairs of ($Pa_{CO_2} - PE_{CO_2}$) measurements on one-lung ventilation was 1.1 (0.7) kPa. Even after correction, mean ($Pa_{CO_2} - PE_{CO_2}$) varied from -0.7 to 0.8 kPa; individual extreme values ranged from -1.3 to 1.7 kPa. Variation between patients was found to be greater than variation within patients for both net and corrected differences (*F* ratio = 37.0 and 10.9, respectively); although calculating a corrected difference did reduce variation between patients from a mean square value of 2.44-0.61.

Our findings of an increased ($Pa_{CO_2} - PE_{CO_2}$) in the lateral position which does not change significantly on one-lung ventilation are in agreement with previous reports [2,3]. The wide variation in ($Pa_{CO_2} - PE_{CO_2}$) suggests that the accuracy of estimation of Pa_{CO_2} by monitoring PE_{CO_2} , although improved by the use of a corrected difference, remains questionable during one-lung ventilation.

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CAN ONE PREDICT ARTERIAL P_{CO_2} FROM END-TIDAL P_{CO_2} ?

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When ventilator tidal volume is increased and ventilatory frequency decreased simultaneously, the decrease in alveolar deadspace fraction is greatest in those patients with worst lung function. This paper tests the hypothesis that the arterial-end-tidal P_{CO_2} difference ($Pa_{CO_2} - PE_{CO_2}$) during anaesthesia/controlled ventilation may be estimated non-invasively by observing what happens to end-tidal P_{CO_2} when ventilator frequency and tidal volume are changed.

Twenty-five anaesthetized adults (mean age 65 (SD 10) yr) undergoing coronary bypass surgery were studied. The patients' lungs were ventilated at 20 b.p.m. with nitrous oxide in oxygen via a Servo 900C ventilator. Expired carbon dioxide was measured by a Siemens carbon dioxide analyser 930. The analyser had been calibrated using a test gas whose carbon dioxide content was confirmed by tonometry with the same apparatus (ABL 300) that was used for all blood-gas analyses. At steady state, PE_{CO_2} was noted (mean of five successive values) and arterial blood was obtained for blood-gas analysis. Immediately after blood sampling, the ventilator rate was reduced to 10 b.p.m. and the mean end-tidal P_{CO_2} of the next five breaths was noted.

When the ventilator setting was changed to 10 b.p.m., doubling tidal volume, PE_{CO_2} increased in 17 patients by mean 0.17 (0.14) kPa. In eight patients, it decreased by mean 0.14 (0.11) kPa. There was a linear relationship between the change in end-tidal P_{CO_2} brought about by this manoeuvre and ($Pa_{CO_2} - PE_{CO_2}$) at 20 b.p.m. ($r = 0.60$, $P = 0.001$, see fig. 9); patients whose end-tidal P_{CO_2} increased the most had the greatest ($Pa_{CO_2} - PE_{CO_2}$) values. Mean ($Pa_{CO_2} - PE_{CO_2}$) in the "risers" was 1.06 (0.40) kPa, in "fallers" 0.73 (0.37) kPa ($P = 0.06$).

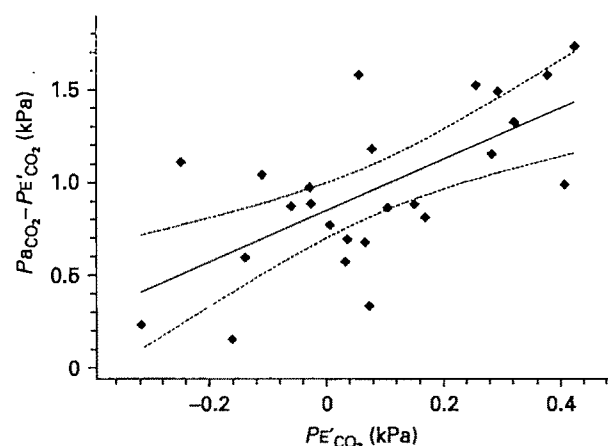


FIG. 9. Relationship between change in end-tidal P_{CO_2} (PE'_{CO_2}) and arterial-end-tidal P_{CO_2} difference ($Pa_{CO_2} - PE'_{CO_2}$).

The spread of ($Pa_{CO_2} - PE_{CO_2}$) values observed in a population of middle-aged/elderly patients undergoing anaesthesia/ventilation is large (0.5-2 kPa). Non-invasive indices, such as preoperative spirometry, age and sex cannot discriminate between patients having large and small ($Pa_{CO_2} - PE_{CO_2}$) values, although smoking habits may be useful [1]. The results suggest that it may be possible to identify those patients who have greatly increased ($Pa_{CO_2} - PE_{CO_2}$) values by a simple non-invasive manoeuvre.

The range of changes in PE_{CO_2} seen here is probably due to differences in *V/Q* spread. In patients with poor lung function, who have a large *V/Q* spread, the change to large tidal volume ventilation improves ventilation of small *V/Q*, large P_{CO_2} regions; PE_{CO_2} increases. In efficient lungs in which *V/Q* spread is small,

the manoeuvre simply generates more carbon dioxide at a smaller partial pressure; PE_{CO_2} decreases.

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POSTER PRESENTATIONS

MONITORING NEUROMUSCULAR BLOCK: COMPARISON OF THE MINI-ACCELOGRAPH WITH A MECHANOMETRIC METHOD

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Accelerometry is a new method of measuring neuromuscular block which may be particularly suitable for routine clinical monitoring. The mini-accellograph, a compact version of the accellograph, has not been compared previously, with a force transducer technique for accuracy and drift during train-of-four (TOF) monitoring.

Thirteen ASA I patients undergoing elective lower limb surgery received a standard anaesthetic comprising thiopentone and enflurane and nitrous oxide in oxygen, with spontaneous ventilation via a laryngeal mask. When stable anaesthesia was established, the transducer of the mini-accellograph was applied to one thumb and the force transducer of a Myograph 2000 was applied to the contralateral thumb. The ulnar nerves were stimulated at the wrists with TOF stimuli delivered at 12-s intervals by identical Myotest stimulators via ECG electrodes. Both forearms were insulated to maintain skin temperature. When control recordings were stable, atracurium or vecuronium was administered in a dose sufficient to produce approximately 95 % twitch depression. Mechanical ventilation was then commenced.

At completion of surgery, neostigmine $40 \mu\text{g kg}^{-1}$ with atropine was administered if necessary to antagonize residual block. Monitoring was continued until the TOF ratio exceeded 0.7 and the first response of TOF had achieved a plateau, at which time enflurane was discontinued. The mini-accellograph and myograph were compared with respect to depression of the first response of the TOF (T_1/T_0) and the TOF ratio, using the method of Bland

and Altman [1], the matched pairs Wilcoxon signed rank test and the unpaired t test. A P value < 0.05 indicated a significant difference.

In nine patients the control TOF ratio measured using the mini-accellograph exceeded 1.0 (median 1.1, range 0.97-1.31) because the first response was smaller than the subsequent three. This was not observed in the myograph recordings. During onset, TOF fade at 50 % and 75 % twitch depression was less pronounced using the mini-accellograph. Using the method of Bland and Altman, the magnitude of the variation in the difference between the TOF ratios, as measured by the myograph and mini-accellograph at 50 % and 75 % twitch depression during onset and 25 % twitch depression during recovery was such that the methods cannot be used interchangeably (table IX).

During onset, the discrepancy between the two methods tended to increase to a maximum at moderate block (33-66 %) before decreasing to a minimum at profound block. The mean differences between the two methods at 33-66 % block were greater than at 0-32 % or 67-100 % block ($P < 0.01$).

During onset, the mini-accellograph underestimated block in eight patients and overestimated it in two patients. At moderate block, the mean difference in T_1/T_0 (myograph *vs* mini-accellograph) was 0.23 (range -0.46 to 0.25).

In contrast, during recovery, the mini-accellograph overestimated block in six patients and underestimated it in two patients. For the majority, the difference between the myograph and mini-accellograph was maximum during moderate block (mean T_1/T_0 difference 0.16, range -0.36 to 0.43).

Drift greater than 10 % of control occurred in three recordings with the myograph and eight recordings with the mini-accellograph.

In conclusion, there are clinically significant differences between the two methods of measuring TOF ratio during onset and offset of neuromuscular block. Differences in single twitch depression appear to become significant clinically only during moderate block. Drift may be a greater problem with the mini-accellograph.

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ACTIONS OF ALPHA₂ ADRENOCEPTOR AGONISTS ON NORADRENALINE RELEASE IN THE RAT LOCUS COERULEUS

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Alpha₂ adrenoceptor agonists have been used as adjuncts to reduce anaesthetic requirements and the selective agonist dexmedetomidine may even have anaesthetic properties. Although the site of action has been suggested to be the locus coeruleus [1], the effects of alpha₂ agonists on noradrenaline efflux in the locus coeruleus have not been studied.

In this study, fast cyclic voltammetry (FCV) [2] was used to

TABLE IX. Mean (SD) differences between the myograph and mini-accellograph in assessing TOF ratios

TOF ratio	Difference
50 % block, onset	-0.25 (0.42)
75 % block, onset	-0.25 (0.48)
25 % block, recovery	-0.005 (0.28)

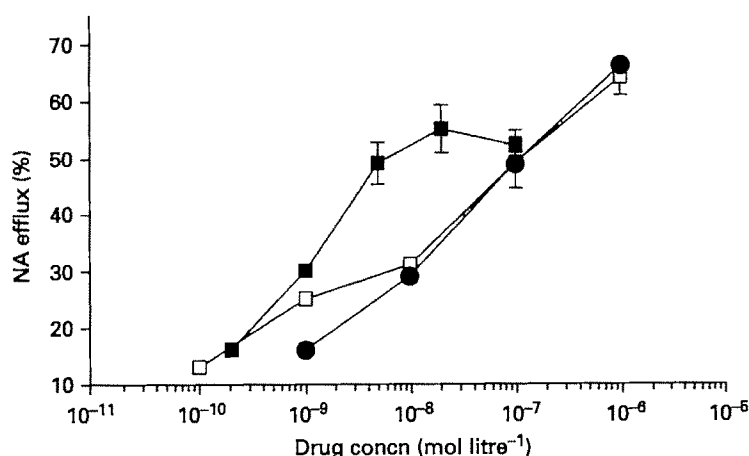


FIG. 10. Mean (SD) inhibition of noradrenaline (NA) efflux by dexmedetomidine (■), clonidine (□) and bromoxidine (●).

construct concentration-response curves for inhibition of noradrenaline efflux by the α_2 agonists dexmedetomidine, clonidine and bromoxidine. In FCV, a voltage passed across a recording electrode causes oxidation and reduction of adjacent catecholamines (in this case noradrenaline efflux into the ECF from the synaptic cleft). Each catecholamine has characteristic oxidation and reduction peaks (for noradrenaline, oxidation occurs at +685 mV and reduction at -220 mV *vs* silver-silver chloride). The speed of the FCV method means that release and reuptake events may be measured in real time and quantitative pharmacology is possible. Experiments were conducted in 350- μ m slices of rat locus coeruleus superfused with oxygenated artificial CSF at 32 °C. Bipolar tungsten stimulating electrodes were placed 200 μ m from a carbon fibre microelectrode at a depth of 80 μ m. Noradrenaline efflux was evoked using trains of 20 pulses (0.2 ms, 10 mA) applied every 5 min and monitored using FCV at the following variables: scan rate 480 V s⁻¹, scan range -1.0 to +1.4 V *vs* silver-silver chloride, interscan interval 500 ms. The short stimulus train minimizes endogenous autoreceptor activation and thus enables agonist effects to be seen.

Noradrenaline efflux was stable for at least a 3-h control period (mean 100.3 (SEM 5.8) %, after 3 h). The α_2 agonists inhibited noradrenaline efflux in the locus coeruleus in a concentration-dependent fashion and the actions were antagonized by the α_2 antagonist atipamezole 1 μ mol litre⁻¹. Calculated EC₅₀ values were 1.1 (0.3) nmol litre⁻¹ for dexmedetomidine, 17 (10) nmol litre⁻¹ for clonidine and 17 (7) nmol litre⁻¹ for bromoxidine. The concentration-response curves were shallow, resulting in large errors in the values. Maximum observed inhibition of noradrenaline efflux was 59 (3) % for dexmedetomidine, 64 (4) % for clonidine and 68 (2) % for bromoxidine (fig. 10).

The similar maximum reduction of noradrenaline efflux by dexmedetomidine and clonidine is at variance with their different reduction of volatile anaesthetic requirement: 48 %, clonidine and > 90 %, dexmedetomidine [3]. The present results suggest that the α_2 receptor mediating inhibition of noradrenaline efflux in the locus coeruleus is, at least, not the sole site of anaesthetic action for α_2 agonists.

ACKNOWLEDGEMENTS

We thank the Royal Society, the Smithkline Foundation and the Association of Anaesthetists of Great Britain and Ireland for support. We also thank Peter Palij for assistance and helpful discussions.

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A LARYNGEAL MASK-OBTURATOR TO PREVENT ASPIRATION OF GASTRIC CONTENTS

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The laryngeal mask airway does not prevent aspiration of gastric contents, thus its use in failed tracheal intubation during emergency anaesthesia may be hazardous. An oesophageal obturator has been shown to prevent regurgitation during resuscitation [1]. A laryngeal mask-obturator (LMO) is described that can maintain a clear airway without the risk of gastric content regurgitation.

The LMO was prepared by fixing an oesophageal obturator to a standard laryngeal mask. The obturator was formed by modifying a 10-mm internal diameter Portex disposable tracheal tube. The bevel of the tracheal tube was cut and polished to render it atraumatic. The oesophageal cuff had a capacity of 30 ml and was connected to a non-return valve.

After obtaining approval of the Hospital Ethics Committee, the LMO was used in patients of ASA I or II, undergoing body surface surgery. Patients who were grossly obese, had hiatus hernia, were allergic to methylene blue or had an oesophageal disease were excluded. Premedication with oral temazepam 0.3 mg kg⁻¹ was administered approximately 90 min before induction of anaesthesia. Methylene blue has been used to detect regurgitation [2]. A gelatin capsule containing 50 mg of methylene blue powder in lactose was taken orally by all patients 10 min before induction of anaesthesia. Presence of the dye in the pharynx indicated regurgitation. Anaesthesia was induced with propofol and direct laryngoscopy was then performed to look for the dye in the pharynx. The LMO was then placed and the number of attempts for its correct placement were noted. Oesophageal and laryngeal cuffs were inflated with 20 ml of air sequentially. Anaesthesia was maintained with enflurane and 35 % oxygen in nitrous oxide and patients were allowed to breathe spontaneously throughout the duration of anaesthesia. At the end of surgery the LMO was removed and direct laryngoscopy was carried out.

Nine males and eight females participated in the study; all were fasted for at least 6 h before operation. Mean age and weight were 36.7 yr and 70.8 kg, respectively. The LMO was *in situ* for a mean duration of 33.8 min. The placement of the LMO was easy and was accomplished in one attempt in 13 patients. There was no regurgitation into the pharynx after induction of anaesthesia in any of the patients. Regurgitation was confirmed in three patients at the end of the operation, by identifying methylene blue staining of the oesophageal obturator below the oesophageal cuff. The LMO prevented regurgitation of gastric contents into the pharynx. Six patients complained of sore throat after operation but none required analgesics.

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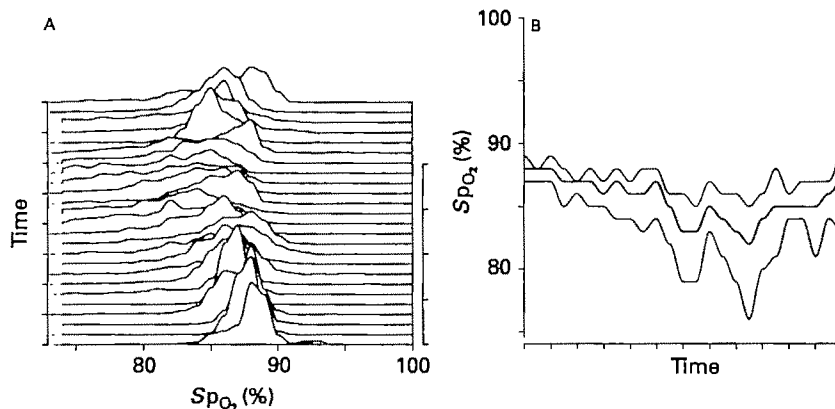


FIG. 11. A: Distribution curves for Sp_{O_2} values *vs* time. B: Simplified version of A, using the 20th, 50th and 80th centiles.

PROCESSING AND PRESENTATION OF DATA FROM LONG-TERM MONITORING OF OXYGEN SATURATION

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The pulse oximeter enables long-term (e.g. overnight) measurement of arterial saturation (Sp_{O_2}). This produces a large quantity of data which when presented as a Sp_{O_2} vs time plot fails to display episodes of desaturation in any detail. We describe a system that allows long-term monitoring of Sp_{O_2} , storing all the data items. This software package produces two diagrams which summarize these data, compressing them to a manageable size while preserving information regarding any significant excursions.

In the first diagram, the data grouped into periods are displayed as a distribution of Sp_{O_2} . These distribution curves are presented as a three-dimensional graph against time (fig. 11A). Each curve in the 12-h study in figure 11A contains 900 data items collected over 30 min. The second diagram shows how the information can be further simplified. For example, in figure 11B we have used the 20th, 50th (median) and 80th centiles of the distribution curve to produce a two-dimensional graph similar to a contour representation of the previous three-dimensional graph (fig. 11A).

Although it is self-evident by visual examination when there is a difference between two intervals, but if the total number of data items is small, the software has the facility to differentiate between two distributions using the chi-square test [1]. This is also used for detecting differences between two subjects over equal time periods.

The only variable undefined in this process is the length of each interval. From our studies [2,3] the ideal interval is apparently related to the time constant of the event under scrutiny. For example, postoperative effects [2, fig. 1] require long intervals (30 min–1 h), whereas haemodialysis [3] is best studied with intervals of 10 min.

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PREDICTION OF PROLONGED POSTOPERATIVE HYPOXAEMIA

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Postoperative hypoxaemia is becoming increasingly recognized to be both prolonged (up to 4 days) and profound ($> 75\%$ Sp_{O_2}) in some patients [1]. It is not cost effective to administer oxygen continuously to every patient for several days after operation. Therefore, it would be useful to be able to use a simple test to predict which patients are likely to experience prolonged hypoxaemia.

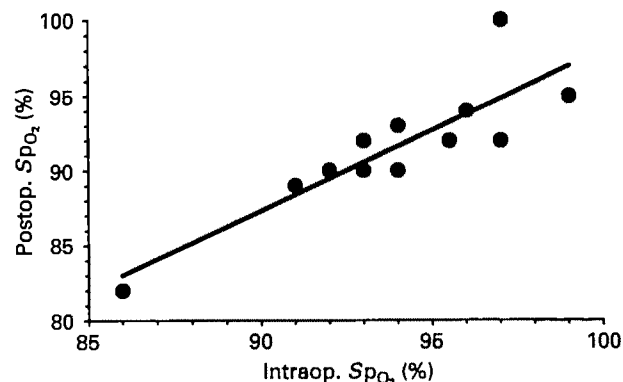


FIG. 12. Relationship between median intraoperative (Intraop.) Sp_{O_2} and median Sp_{O_2} values on the first and second nights after operation (Postop.)

Continuous Sp_{O_2} measurements were made in 12 patients breathing oxygen at an interpolated partial pressure of 21 kPa before, during and after major abdominal surgery. The Sp_{O_2} data were displayed as a series of plots of distribution of Sp_{O_2} as described previously [1]. There was poor correlation between preoperative Sp_{O_2} values and either the median Sp_{O_2} on the first or the second night after operation. In contrast, there was good correlation between intraoperative Sp_{O_2} ($PI_{O_2} = 21$ kPa) and both the median Sp_{O_2} on the first ($r^2 = 0.61$) and second ($r^2 = 0.75$) nights after operation (fig. 12).

We conclude that patients with a low intraoperative Sp_{O_2} at PI_{O_2} of 21 kPa will be most likely to exhibit prolonged low saturation after operation. In contrast, measurement of Sp_{O_2} before operation is a poor predictor of prolonged postoperative hypoxaemia.

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HALOTHANE, ENFLURANE AND ISOFLURANE INCREASE K^+ STIMULATED EXTRASYNAPTOSOMAL GLUTAMATE ACCUMULATION

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Glutamate is a major excitatory neurotransmitter. It has been implicated in central neuroplasticity [1], and drugs decreasing glutamate release decrease anaesthetic requirements [2].

Female Wistar rat cortical synaptosomes were prepared using the method of Dunkley and colleagues [3] and suspended in Krebs-HEPES buffer at a protein concentration of 5 mg ml^{-1} . After equilibration with either air or air containing 2.4 (rat) MAC of halothane, enflurane or isoflurane at 37°C , the synaptosomes were depolarized with KCl 40 mmol. Immediately, and after 10 min, aliquots were sedimented and the supernatant assayed for glutamate content. In the assay, L-glutamate was oxidized to 2-oxoglutarate by glutamate dehydrogenase, a reaction coupled to production of NADH. The increase in NADH was measured by following the increase in evoked fluorescence. Statistical analysis was with paired and unpaired analysis of variance, as appropriate.

Supernatant glutamate accumulation after K^+ -induced depolarization was increased significantly in the presence of all three volatile anaesthetics compared with air (fig. 13). No significant differences were observed between the three volatile agents.

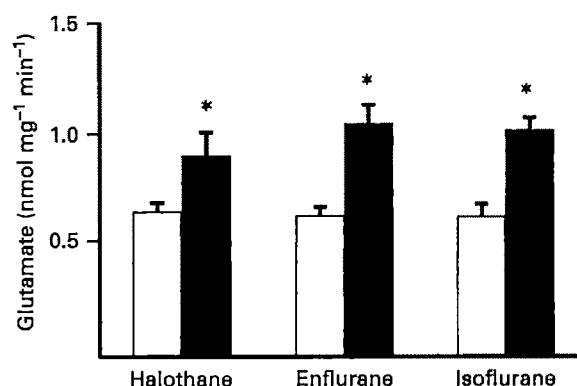


FIG. 13. Extrasynaptosomal glutamate accumulation evoked by depolarization in the presence and absence of volatile anaesthetics (■) and air alone (□). Data are mean and SEM, $n = 10$. * $P < 0.05$ compared with air.

This study suggests that K^+ stimulated extrasynaptosomal glutamate accumulation is enhanced in the presence of the volatile anaesthetics studied. These results are not consistent with a major presynaptic mechanism of anaesthesia involving glutamate.

ACKNOWLEDGEMENTS

C.M.W. is an intercalated MRC B.Sc. student, and R.G. is a *British Journal of Anaesthesia* Research Fellow.

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INFLUENCE OF THE DAY OF THE MENSTRUAL CYCLE ON THE INCIDENCE OF POSTOPERATIVE NAUSEA AND VOMITING

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In women undergoing gynaecological laparoscopy, it has recently been shown that the incidence of postoperative nausea and vomiting varies markedly with the menstrual cycle, with a four times greater risk during the menses [1]. To establish if this is true of patients undergoing other surgical procedures, we have prospectively studied 195 premenopausal women presenting to our institution for third molar extractions.

All patients received premedication with papaveratum 0.2 mg kg⁻¹ and hyoscine 4 µg kg⁻¹. Induction was with thiopentone 4-6 mg kg⁻¹ and intubation was facilitated by suxamethonium or a non-depolarizing agent. Anaesthesia was maintained with either enflurane or isoflurane and nitrous oxide in oxygen. At induction, all patients received prochlorperazine 0.17 mg kg⁻¹ i.v. Patients were followed-up until discharge 4.5 h after awakening. For analysis, patients were divided by day of cycle into three groups: days 1-8 (pre-ovulatory); days 9-15 (ovulatory) and days 16 to the end of cycle (post-ovulatory). Chi-square analysis (with Yates' correction as appropriate) was used to compare the incidence of nausea and vomiting between each group. Statistical significance was accepted at *P* < 0.05.

The overall incidence of nausea and vomiting was 18.5%. The incidence of postoperative nausea and vomiting was significantly higher on days 9-15 (*P* < 0.05) compared with the incidence on days 1-8 and on days 16 to the end of cycle. When the 95 patients

taking the oral contraceptive pill were analysed independently, the incidence of nausea and vomiting remained significantly greater on days 9-15 (*P* < 0.05). In the 100 patients who were not taking the oral contraceptive pill, the incidence of postoperative nausea and vomiting was greater on days 9-15, but this was not statistically significant (*P* = 0.36) (table X).

TABLE X. Incidence of postoperative nausea and vomiting (%) by day of menstrual cycle and in those taking the oral contraceptive pill (OCP). **P* < 0.05 compared with days 1-8 and days 16 to the end of cycle

	Day of menstrual cycle		
	1-8	9-15	16+
All patients	19	29*	12
Patients on OCP	22	36*	12
Patients not on OCP	18	23	13

In our study, a greater incidence of postoperative nausea and vomiting occurred on days 9-15 of the menstrual cycle in women undergoing third molar extractions. This is in contrast to Beattie and colleagues who found a greater incidence of postoperative nausea and vomiting on days 1-8 of the menstrual cycle after gynaecological laparoscopy [1]. The greatest incidence of postoperative nausea and vomiting in our study corresponds to the greatest concentrations of oestrogens found during the menstrual cycle. It is possible that oestrogens may sensitize the chemoreceptor trigger zone, the vomiting centre, or both, to effect this cyclical variation in female susceptibility to postoperative nausea and vomiting. Further studies measuring the concentrations of oestrogen may clarify this mechanism.

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CORRESPONDENCE

PERIOPERATIVE SEIZURES AND FLUVOXAMINE

Sir,—We recently anaesthetized a healthy, 50-kg, 38-yr-old woman for extraction of wisdom teeth as an outpatient. Apart from taking fluvoxamine 200 mg day⁻¹ for depression, her pre-operative assessment was unremarkable.

She received no premedication. Anaesthesia was induced with propofol 150 mg, fentanyl 0.1 mg and droperidol 1.25 mg. After suxamethonium 100 mg, the trachea was intubated with a nasotracheal tube. The lungs were ventilated by a Penlon 200 ventilator and Bain system delivering 66% nitrous oxide in oxygen at a fresh gas flow of 6 litre min⁻¹. Anaesthesia was maintained with 2% enflurane, reducing to 1% during the procedure, which lasted 45 min. No additional neuromuscular blockers were required.

At the end of surgery the pharynx was suctioned and 100% oxygen and doxapram 40 mg administered. Shortly after this, the patient coughed on the tracheal tube, became rigid and began a series of dramatic generalized tonic-clonic movements, with opisthotonos typical of a grand-mal type seizure. She was given midazolam 2 mg and 100% oxygen. The seizure subsided over about 1 min, although she continued to have generalized increased muscle tone with marked ankle clonus which resolved gradually over the next 30 min. The patient was drowsy initially, but thereafter made a full and unremarkable recovery.

Although she was keen to go home that day, she was admitted to the ward for overnight observation. She remained well, with no further neurological symptoms or signs, and was discharged the following morning.

Perioperative seizures in apparently normal patients may be multifactorial and it is clearly not possible to identify one cause with certainty when several agents have been used.

Fluvoxamine maleate is a selective inhibitor of serotonin re-uptake. Such drugs are increasingly being used to treat a wide variety of psychiatric disorders. One reason for their popularity is an apparent relative lack of side effects and toxicity in overdose compared with the tricyclic antidepressants or monoamine oxidase inhibitors [1]. Among the listed side effects of fluvoxamine are agitation, tremor and convulsions. The drug reduces the threshold for convulsion and is contraindicated in patients with a history of epilepsy [2].

The ability of enflurane to induce epileptiform activity and grand-mal seizure patterns is well established and has been reviewed comprehensively [3]. The situation with regard to propofol is more controversial, with reports of both pro- and anticonvulsant activity in some circumstances [4]. The Committee on Safety of Medicines have received 54 reports of convulsive episodes (including generalized convulsions, myoclonic jerks, opisthotonos) associated with propofol [C. S. M.—personal communication].

The analeptic agent, doxapram, is sometimes used at the end of anaesthesia to stimulate ventilation and accelerate recovery from the effects of volatile agents without antagonizing analgesia [5, 6]. A modest dose (less than 1 mg kg⁻¹) was administered slowly to our patient, as surgery had been more prolonged than anticipated and ended rather unexpectedly. The drug has central stimulant activity, especially in large doses, and its use is not advised in known epileptics [7]. However, the Adverse Reaction Drug Analysis Information Service yielded only two reports of convulsions associated with doxapram in a 28-yr period [C. S. M.—personal communication].

It seems possible that this patient suffered an epileptiform seizure as a result of receiving a proconvulsant anaesthetic sequence against a background of an antidepressant which decreases the threshold for convulsion. As the 5-hydroxy-tryptamine receptor inhibitors are a relatively new group of antidepressants which are increasing in popularity, it is important for us to be aware of possible interactions with anaesthetic agents. Any further reports of similar problems would be most helpful in establishing the role (if any) of fluvoxamine in the events that we witnessed.

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TRACHEAL INTUBATION WITHOUT NEUROMUSCULAR BLOCK

Sir,—Drs Davidson and Gillespie [1] have shown that tracheal intubation is frequently possible in healthy patients, using propofol with alfentanil and lignocaine, without the use of neuromuscular junction blocking agents. Their induction regimen included alfentanil given 1 min before induction with propofol. Both propofol [2] and alfentanil [3] have the capacity to produce apnoea and do so significantly in combination [4].

The authors do not state if the patients' lungs were pre-oxygenated, but they noted that monitoring included continuous pulse oximetry, although they did not record the results of oximetry. They did not comment on the presence or duration of apnoea associated with this technique. It is documented that desaturation may occur at induction of anaesthesia [5, 6]; when administering drugs that produce apnoea in association with anaesthesia or in patients in whom intubation may be difficult, preoxygenation should be used routinely.

It would seem sensible to preoxygenate the lungs of all patients before intubation attempts with Davidson and Gillespie's technique. It is also worth noting that one of the advantages of intubation without neuromuscular paralysis is that a patient may breathe spontaneously after intubation; if the technique is associated with prolonged apnoea, this advantage is lost.

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Sir,—The lungs of all patients in our study were preoxygenated before induction. Indeed, it is the routine practice of both authors to preoxygenate for all patients undergoing tracheal intubation. The arterial oxygen saturation was monitored continuously

throughout the procedure. No significant changes from baseline were noted in any of the groups after either induction or intubation.

With regard to the occurrence and duration of apnoea after induction: although several patients continued to breathe spontaneously, most became apnoeic after administration of the combination of propofol, alfentanil and lignocaine. However, the study was designed to determine the feasibility of laryngoscopy and tracheal intubation using this regimen, and consequently measurement of apnoeic period was felt to be of lesser importance in the first instance. Having demonstrated that this technique can provide adequate intubating conditions in up to 93% of patients, we are at present undertaking additional studies to assess the duration of apnoea using this approach.

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HEPARIN CONTAMINATION OF SPECIMENS COLLECTED FROM ARTERIAL CANNULAE

Sir,—The investigation by Haynes and colleagues [1] of the accuracy of coagulation studies using arterial cannula samples raised the interesting question of contamination with heparin flush.

They cited Lew, Hutchinson and Lin [2], stating that accurate results may be obtainable when 5 ml of blood is withdrawn and discarded before sampling. Consequently, they withdrew 5.6 ml of blood before collecting the sample.

In unpublished observations made on cardiothoracic surgical patients in theatre before heparinization, we found that, after the deadspace was discarded, sampling from a proximal port at 15 cm from the cannula necessitated 3 ml of blood to be wasted before achieving a "clean" sample. If blood was sampled at 150 cm from the cannula, deadspace plus 7 ml of blood had to be wasted to give an uncontaminated sample. We also used heparin 2 iu ml⁻¹ in our flush; the coagulation tests in this small study were activated partial thromboplastin time and thrombelastography.

In the light of Haynes and colleagues' conclusion that "a blood sample from an arterial cannula may give clinically misleading information because of contamination with small amounts of heparin", it would seem inevitable that contamination would occur if sampling was made at a distance of about 200 cm from the cannula. However, their point of sampling was not stated.

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Sir,—The choice of reagent used for measuring activated partial thromboplastin time has a significant bearing on the degree to which heparin contamination prolongs the result [1]. This was not considered adequately by Lew, Hutchinson and Lin [2]. The reagents used in our study are used widely in haematology laboratories in Great Britain, and we feel that the results of our study are of direct relevance to clinicians in this country. Dr Main does not mention the reagent used in his unpublished work. The samples in our study were taken 15 cm distant from the arterial cannula.

Thrombelastography is a useful technique for rapid diagnosis of a coagulopathy, but its interpretation is open to a degree of subjectivity. At present, its use remains confined to specialized units such as those involved in the provision of liver transplantation.

We believe that, unless shown otherwise in carefully conducted studies, the only practical solution to the problem is to accept the results of our work! That is, that heparin contamination in minute

amounts may occur unpredictably in blood samples taken from arterial cannulae.

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EFFECT OF PEEP ON HYPERINFLATION

Sir,—We congratulate Dr Tan and colleagues on their well designed and informative study concerning the effects of PEEP in patients with obstructive airways disease [1].

While we have no argument with their findings or the conclusions drawn, we have noted a fundamental statistical error.

Two methods were used to measure intrinsic PEEP; these were airway pressure at the onset of inspiratory flow (Paw,0) and airway pressure obtained with expiratory port occlusion at end-expiration (Poc). The authors then calculated a correlation coefficient between the two variables and concluded they were "in reasonable agreement ($r = 0.87$)". Having reached this conclusion, they referred to only one measurement (Poc) in further analyses.

The misleading use of correlation coefficients in the comparison of two measurement techniques has been highlighted by Bland and Altman [2]. Regression analysis is used to predict one variable from another (i.e. y from x), whereas correlation measures the strength of a relation between two variables. This is not the same as agreement, nor does it describe accuracy.

Bland and Altman then described a method of measuring agreement between two measurement techniques. This involves using the average of the two measurements as the best estimate of the true value, and then obtaining the difference between this average and each of the measured values. A mean difference and SD are then obtained. These are the "bias" and "limits of agreement" of the measurement techniques.

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Sir,—The method of estimation of PEEPi was not a prime consideration of our study, but merits further comment. Paw,0 is a measurement of end-expiratory alveolar pressure under dynamic conditions, while Poc is measured under static conditions. To average the two variables to obtain the "best estimate of the true value", as suggested, would be misleading. Nonetheless, the data have been examined by the method of Bland and Altman [1] and the two measurements are in "reasonable" agreement. The bias mean difference is 0.06 (SD 0.17) kPa (fig. 1).

Poc (as a measure of whole-lung static PEEPi) was used in our study to analyse the data of hyperinflation and effects of PEEPe as this is the standard method for the estimation of PEEPi [2] and allows comparison with previous work. Poc has a high degree of reproducibility as the measurement is dependent on the resolution of the pressure transducer alone, whereas Paw,0 depends on the resolution of pressure, flow and the rate of data acquisition.

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1. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307–310.

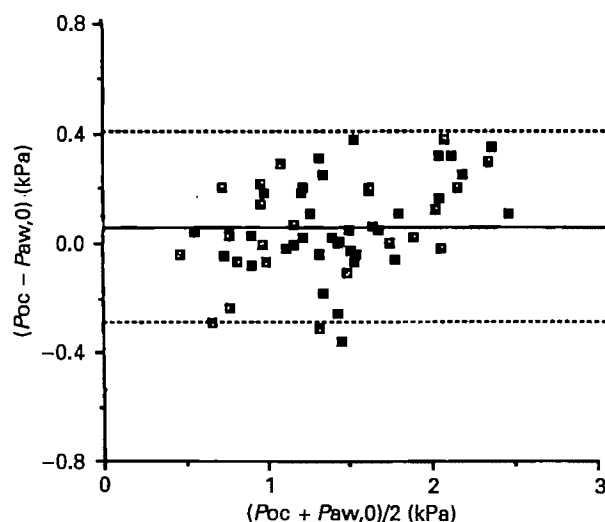


FIG. 1. Bland and Altman comparison of the two methods of measurement of PEEP. Horizontal lines: continuous = mean; dashed = SD.

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PREMEDICATION BEFORE CARDIAC SURGERY

Sir,—We were interested in the article by Pollock and Kenny concerning the effects of a nocturnal dose of lorazepam on oxygen saturation (Sp_{O_2}) during the night preceding cardiac surgery [1]. We have compared the effect of lorazepam and temazepam, given the night before coronary artery bypass surgery, on Sp_{O_2} during that night. Informed consent was obtained from 30 patients: none had a history of chest disease; all had $Sp_{O_2} \geq 96\%$ when awake. They were allocated randomly to receive lorazepam 2-4 mg or temazepam 20-40 mg. Sp_{O_2} was monitored with an Ohmeda Biox 3700, the digital output of which was sampled using a BBC Master Series computer. Data were recorded whenever the Sp_{O_2} decreased to less than 92%, allowing the duration and severity of any period of desaturation to be estimated. Recordings were made throughout the night, until the patients received the i.m. component of their premedication the next morning.

There were no significant differences between lorazepam and temazepam in the number of episodes of desaturation to less than 92%, the duration of the episodes, mean Sp_{O_2} during one of these periods or the smallest Sp_{O_2} recorded. There was one striking difference between the groups: quality of sleep. Those in the lorazepam group usually slept through until morning, but those given temazepam complained of early, frequent waking and difficulty getting back to sleep again.

In conclusion, our findings confirm those of Pollock and Kenny that, in patients about to undergo cardiac surgery, lorazepam sedation does not cause alarming episodes of desaturation. Moreover, we have shown that desaturation associated with lorazepam compares favourably with that associated with temazepam, a drug which is generally considered a milder sedative and which produced poorer sleep in our study. These findings have affected our practice: we now use lorazepam more widely, even in the higher risk patients.

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PERIPHERAL NERVE INJURY CAUSED BY INJECTION NEEDLES

Sir,—One reason for the study by Rice and McMahon [1] seems to be to question an earlier experimental study on the acute effects of needle point trauma by Selander, Dhunér and Lundborg [2]. When criticizing that study, Rice and McMahon unfortunately made inappropriate comparisons. They studied the amount and long-term pathology of injuries in the rat monofascicular sciatic nerve after penetrating it with long- (12°) or short- (27°) bevel injection needles, whereas Selander's group aimed primarily at the frequency and acute morphology of nerve fascicle lesions after piercing the rabbit multifascicular sciatic nerve with a long- (14°) or short- (45°) bevel needle.

The experiments by Selander, Dhunér and Lundborg [2] showed that the nerve fascicles easily slid or rolled away from the needle point both *in vivo* and *in vitro*, especially when the 45° bevel was used. This led to a significantly smaller frequency of fascicle injury after piercing the nerve with the 45° or short-bevel (SB) compared with the 14° or long-bevel (LB) needle.

When a needle hits a nerve, paresthesiae are elicited and the (awake) patient normally reacts to this. The paresthesia informs the anaesthetist that the needle is in close contact with the nerve and that, if advanced further, the needle may injure the nerve, and it seems that most anaesthetists agree with this view [3-6]. Selander, Edshage and Wolff [7] and Plevak, Linstromberg and Danielsen [8] found that the use of a paresthesia searching technique for axillary plexus blocks (with long-bevel needles) increased the frequency of post-anaesthetic nerve injuries compared with a non-paresthesia technique. The use of short-bevel needles may press or push the nerve away, thereby giving the patient and the informed anaesthetist more time to react to a paresthesia before the needle penetrates and possibly injures the nerve.

Rice and McMahon [1] used a short-bevel needle with a bevel angle of only 27°. Their short-bevel needle was also 13% thicker than the 12° long-bevelled needle used. To injure the single fascicle of the rat sciatic nerve "the needle was introduced into the exposed nerve at mid thigh level at an angle of 45° and left undisturbed for 10 min". This would not be possible unless the animal or patient was deeply asleep! It apparently implies that each nerve was severely injured by the needle, and under such unconventional conditions it may not be surprising that the 27° needle caused more severe lesions than the 12°. However, as seen in table I of their paper, the acute lesion on day 1 was worst after the nerve was pierced with the transversely orientated long-bevel needle, as in Selander's study.

In the histological study, something termed "intraneural disruption" was assessed and "this was defined as the degree of disruption to the internal elements of the nerve and included such factors as the reaction at the site of injury, the gliosis... scored on a 0-5 scale..." (my italics). Other morphological changes studied were "evidence of axonal degeneration" and "evidence of disorganized regeneration of fibres", both without closer description and scored only on a present or absent basis—that is, 1-100% = yes; 0 = no. In order to create a global assessment of nerve fascicle injury (which had sufficient security to enable statistical analysis...) ..., "observations of epineural disruption" (my italics) were included, also without a word of definition. No microscopic pictures were included to illustrate these changes.

The statistical handling of these histopathological features was puzzling: "...a table of high and low score point allocation was constructed". The summed injury score was "allocated to the low score table if the epineural or intraneural score was less than 2.5 or when no evidence of axonal degeneration or disorganized fibre regeneration was observed. The converse applied to point allocation to the high score group" (my italics). It seems as if this system makes a variety of interpretations and a high degree of bias possible.

In their Discussion, Rice and McMahon did not discuss the relevance and validity of their results. The authors seem not to understand the fundamental differences in study design and needle penetration techniques between their study and that of Selander, Dhunér and Lundborg. In the latter study, only a quick needle penetration of a multifasciculate nerve was made, to study the frequency of fascicular injury, whereas Rice and McMahon stated: "The rat sciatic nerve used in our study consists of only one fascicle at mid-thigh level and nerve (fascicle) penetration was ensured in all cases". How, then, is it possible to conclude that

"The results of these experiments suggest that when nerve fascicles are impaled by commercially available injection needles (of similar diameter), lesions occur less frequently—if they are induced by an LB needle compared with an SB needle" (my italics). Obviously, *all nerve fascicles* were deliberately injured with the test needles!

The clinical significance of paresthesiae was overlooked also when Rice and McMahon could not confirm the recommendation of Selander, Dhunér and Lundborg to use a 45°-bevel needle in clinical anaesthesia. Instead, they suggested that "there may be grounds for recommending the use of LB needles, especially if the bevel is aligned parallel to the nerve fibres". This is probably true if one deliberately penetrates the nerve with the needle before injecting the local anaesthetic, but such a technique cannot be recommended, as an intraneural injection severely increases the risk of nerve injury [2, 7, 9, 10].

Fortunately, nerve injuries caused by regional anaesthesia are rare, but even so, they may cause severe and long-lasting suffering for the unfortunate patient and perhaps also for the anaesthetist. It is naturally important that any recommendations concerning methods or techniques for clinical use are based on sufficient clinical experience and basic research.

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Sir,—The aim of our study was not to "question" the much-quoted study by Selander, Dhunér and Lundborg [1], but to complement it by examining different aspects of an animal model, over a time course more appropriate to the natural history of nerve injuries. As Selander points out, there are fundamental conceptual differences between the two papers. He examined the extent to which injury of the perineural covering of nerve fascicles was influenced by bevel design, over a very short period of time. We described the influence of needle bevel upon the severity and consequences of intrafascicular lesions, should accidental fascicular penetration occur. Selander implies that we were unaware of these differences or chose to ignore them. This is quite clearly not the case, as we were at pains to point out several times in our text. Indeed, the major part of the discussion was devoted to alerting the reader to these differences.

We fully agree with Selander that all possible steps, such as avoidance of paraesthetic techniques, should be taken to avoid fascicular injury. Our study examined the effects of bevel design upon the severity and duration course of intrafascicular lesions,

following fascicular penetration. The ability of different bevels to penetrate nerve fascicles (or trunks), or to push them away, in the clinical situation of the non-surgically exposed nerve is, at present, unknown. We shall therefore refrain from making dogmatic recommendations until such evidence is available. However, in answer to Selander's question: we did not measure ease of fascicle penetration, but our impression was that it was, as would be expected, easier to enter the fascicles with the long-bevel needles; these commercially produced needles possess a secondary cutting bevel. We suggest that long- and medium-bevel needles without a cutting bevel (or even "pencil-point" tips [2]) should be investigated as a compromise between the supposed relative difficulty of entry into a nerve fascicle with short-bevel needles and the severe lesions which we have shown to occur should fascicles become impaled with such needles.

The two needles we used were of a similar size and both designs are used commonly in the practice of regional anaesthesia. Our method of impaling the nerves has been used by others investigating mechanical nerve injury [3] and is not dissimilar to that used by Selander in his study. In answer to his point regarding the angle of entry, this was 45° to the nerve. We decided to use this angle having examined the techniques of others investigating mechanical fascicular injury [3]. Why did Selander's group choose 90° [1]?

We dispute Selander's postulate that fascicular penetration is possible only in deeply anaesthetized animals or humans. We have both undergone many fascicular penetrations of the ulnar nerve during the course of microneurography recordings with a 30-g recording electrode. Fascicular penetration (as demonstrated by microneurographic recordings) is surprisingly difficult to predict on symptomatic grounds alone [4]. Also, many anaesthetists perform regional anaesthesia in patients under general anaesthesia.

With regards to the comments on our summed nerve injury scores. The whole point of this exercise was to reduce the inevitable bias and observer error of single histological features. We displayed most of the individual histological data in table I, so that the reader might draw his own conclusions. The epineural scores were excluded to avoid repetition, as they were very similar to the intraneural scores; they have been published elsewhere [4]. The sensitivity of the summed nerve injury scores was shown clearly, when the same technique was used to investigate the more minor injuries associated with microneurography [4].

Several representative photomicrographs were submitted with the original manuscript, as we also felt that the reader should be able to form a visual impression of the type of lesions being discussed. They were omitted from the published version at the suggestion of the editor.

Selander has misunderstood our use of the term "frequency". Of course, all fascicles were impaled; this was the aim of the study. The term frequency was used to describe the incidence of observed lesions following fascicle injury.

In the discussion we stressed the various differences between the study by Selander, Dhunér and Lundborg [1] and ours. We referred to this well known study because, despite the obvious fundamental difference from our paper, it is the only relevant publication in this field. We did not attempt to make any clinical recommendations from our results, but demonstrated that, should accidental fascicle penetration occur, the lesions observed following the use of short-bevel needles are more severe and take longer to repair than those from long-bevel needles. We stated that a great deal more basic and clinical work needs to be performed before definitive statements can be made regarding the design of needles for use in regional anaesthesia. We most certainly do not advocate the practice of intraneural injection! Clinical practice has already been changed once following a single, limited, animal study which has never been backed up by clinical research. We are reluctant to make further recommendations until a great deal more basic and clinical research has been performed in order to elucidate this issue.

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REDUCTION OF DOPAMINE RELEASE AND POSTOPERATIVE EMESIS BY BENZODIAZEPINES

Sir,—We read with interest the study by Takada and colleagues [1] on the effects of midazolam and flunitrazepam on the release of dopamine from rat striatum.

Recently, we postulated that benzodiazepines may reduce the synthesis, release and postsynaptic effect of dopamine centrally [2]. This action at the chemoreceptor trigger zone is a possible mechanism for the antiemetic effect of benzodiazepines [2]. Anxiolysis mediated by benzodiazepine binding to the GABA_A-benzodiazepine receptor complex may also contribute to the antiemetic effect by reducing the psychic input to the vomiting centre [2].

The study by Takada and colleagues [1] supports the hypothesis that benzodiazepines reduce dopamine release centrally. However, they suggest that the effect is mediated by benzodiazepine binding to the GABA_A-benzodiazepine receptor complex, causing inhibition of dopaminergic neuronal activity. The reason given for this conclusion is that the benzodiazepine effect on dopamine release was abolished by flumazenil.

It has been shown that flumazenil is also an adenosine antagonist [3]. Benzodiazepines block the re-uptake of adenosine and some of their central effects may be explained by an increased adenosine action [3]. Adenosine, a central neuromodulator, has been shown to inhibit nigrostriatal dopamine release by an adenosine-1-receptor mediated effect [4].

The alternative explanation for the reduced release of dopamine after administration of midazolam and flunitrazepam is that benzodiazepines block the re-uptake of adenosine, causing an adenosine-mediated reduction in dopamine release [2]. This effect can be blocked by flumazenil acting as an adenosine antagonist.

The regional differences in the effects of midazolam and flunitrazepam found by Takada and colleagues [1] may reflect the differing activities of the drugs on adenosine neuromodulation, the GABA_A-benzodiazepine receptor complex or both. Obviously, further studies are required to clarify this problem.

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1. Takada K, Murai T, Kanayama T, Koshikawa N. Effects of midazolam and flunitrazepam on the release of dopamine from rat striatum measured by *in vivo* microdialysis. *British Journal of Anaesthesia* 1993; **70**: 181-185.
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Sir,—Drs Di Florio and Goucke suggest that our results could be explained by an inhibitory effect of midazolam and flunitrazepam on adenosine uptake [1], because adenosine also inhibits dopamine release [1]. Given the lack of selectivity of benzodiazepines, benzodiazepine receptor antagonists [2-4] and the ability of benzodiazepines to modify adenosine A₁ and A₂ receptors [5], the suggestion of Drs Di Florio and Goucke merits further consideration. However, it is possible that the effect of adenosine on dopamine release may still be mediated by the GABA-B₂ receptor

chloride channel because of the close association of some adenosine receptors with this receptor complex [6].

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ONSET OF BLOCK WITH VECURONIUM AND BODY MASS INDEX

Sir,—I was interested in the demonstration by Drs Gill and Scott [1] that the onset of vecuronium 0.1 mg kg⁻¹ was faster after induction with etomidate compared with thiopentone or propofol. I am concerned, however, that excessively heavy patients do not appear to have been excluded from the study.

It is stated that weights ranged from 50 to 110 kg. Body mass index is the weight divided by the height squared. According to a standard nomogram [2], in order to escape classification as "obese", a 110-kg male would have to be more than 190 cm (6'3") tall. Only if his height were to exceed 210 cm (6'11") would his weight be accepted as normal!

It has been shown that increasing the dose of vecuronium from 0.1 to 0.15 mg kg⁻¹ markedly decreases onset time in patients within 20% of ideal body weight [3]. The administration of a neuromuscular blocker to an obese patient on an mg kg⁻¹ basis constitutes an overdose. If the patient weighing 110 kg belonged to the etomidate group, the mean onset time would have been spuriously reduced.

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Sir,—Standard teaching, for many years, has been to prescribe drugs on an mg kg⁻¹ basis, although this may not be the most appropriate scale to use, especially in obese, burns and any patient with an altered basal metabolic rate. However, weight is the most available source of information in the routine ward setting upon which to base an appropriate dose of a drug.

We agree that the use of this scale may have allowed a patient who was obese to have received a relative overdose of vecuronium.

However, none of the three groups differed statistically with regard to the weights of patients. Those in the etomidate group had a mean weight of 74.2 kg (range 50–90 kg). Therefore it is unlikely that this variable could have been a confounder in our results.

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A TRACHEAL TUBE PROTECTOR TO PREVENT KINKING

Sir,—A tracheal tube hidden under surgical drapes may be inadvertently occluded. For example, during surgery on the cervical region, the arms of surgical staff may involuntarily compress the tube. In such situations, the patient's upper teeth act as the fulcrum of a lever, leading to a kink in the tracheal tube at the lips. To solve such problems, special tracheal tubes such as the RAE preformed tube and a reinforced tube have been developed. However, these tubes are not used frequently and they are cumbersome for tracheal intubation and suction.

We were faced recently with difficulty in ventilation of the lungs

of a patient because of kinking of the tracheal tube during anterior fusion of the cervical vertebra. To solve this problem, we devised a protector using a modified anaesthetic mask. We cut off part of the plastic dome of an infant mask (size 1) to create a square hole approximately 15 × 15 mm to fit a tracheal tube (fig. 1). After tracheal intubation, the tube was mounted on the concave portion of the mask and then connected to an anaesthesia circuit. The mask was fixed to the face with adhesive tape (fig. 2). This simple equipment prevented the tracheal tube from kinking because of the weight of the drapes or the surgeon's arm. It was not an obstacle to surgery because of its small size.

We now routinely use this protector for surgery in the cervical region.

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FIG. 1. The tube protector. The edge of the plastic dome of an anaesthetic mask was cut to form a square hole approximately 15 × 15 mm to fit tracheal tube.



FIG. 2. The protector in clinical use (volunteer).

A SIMPLE INDICATOR FOR IDENTIFYING THE EXTRADURAL SPACE

Sir,—Recognizing when the point of the needle pierces the ligamentum flavum and enters the extradural space is a critical step in performing extradural anaesthesia. Forty-three years ago, Macintosh proposed an ingenious device for identifying this space, based on the existence of negative pressure in the extradural space [1]. This indicator comprises an inflated rubber balloon attached to a Tuohy needle. When the tip of the needle enters the extradural space, the balloon deflates abruptly. However, the materials for this device are not readily available, and its assembly is awkward.

Based on the same principal, we have developed a simple device using common, readily available materials that are found in all operating theatres: a Foley catheter, a stopcock and a needle for i.m. injections. The extradural needle is attached to the female end of the stopcock using the male connector included in the set (Minipack, Portex). The Foley catheter Fr16 is cut transversely approximately 7 cm from its distal end and is attached to the side connection of the stopcock. The i.m. needle (21-gauge) is inserted into the narrow lumen of the Foley catheter leading to the balloon, and a syringe with a few millilitre of air is attached to this needle. The stopcock valve is adjusted to connect between the syringe and the Foley catheter, and the balloon on the catheter is inflated. After the needle is placed in the spinal ligaments, the stopcock valve is turned to seal the air in the balloon and open the connection between the Foley catheter and the extradural needle. This device is steadily advanced through the tissues with constant pressure. As soon as the needle enters the extradural space, the balloon abruptly deflates, indicating the location.

For correct and effective use, the device must be sealed so that the deflation of the balloon is abrupt. Furthermore, confirmatory tests for extradural puncture, including an aspiration test, must be carried out.

This technique is very useful for identifying the extradural space, especially at the thoracic and cervical levels, where a positive hanging drop sign is not always an infallible index of correct extradural localization [2].

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HAEMODYNAMIC EFFECTS OF PROPOFOL

Sir,—We read the article by Lindgren and colleagues [1] comparing propofol and thiopentone and congratulate the authors for a fine study on the use of propofol and its haemodynamic

effects. The haemodynamic data are consistent with those found in some studies by other investigators and were cited as such [2-4]. However, in the authors' discussion they stated that "the reduction in cardiac index was predominately caused by a reduction in systemic vascular resistance" and cited work by Lippmann and colleagues [2], amongst others. This particular reference is incorrectly quoted. The papers by Lippmann and colleagues did not state that the reduction in cardiac index was caused by a profound decrease in systemic vascular resistance, but was caused by propofol itself, which may be regarded as a direct myocardial depressant. The data were as follows: MAP reduced $23 \pm 12\%$, CI reduced $18 \pm 13\%$, LCWI $35 \pm 16\%$ and LSWI $35 \pm 18\%$. SVR, PVR and PCWP remained essentially normal [2]. Other investigators have also found that propofol, whilst affecting SVR, was a myocardial depressant [3, 5]. We wish to bring to the attention of Dr Lindgren and colleagues the importance of accurate quoting of references, as highlighted recently in the North American literature [6].

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Sir,—There are several well conducted studies which show that systemic vascular resistance (SVR) decreased after propofol [1-14]. Most interestingly, Rouby and others [11] demonstrated that SVR decreased after propofol in patients with artificial hearts to a greater extent than after thiopentone. In this situation, cardiac output remains constant. Furthermore, plasma renin activity increased after propofol, suggesting peripheral vasodilatation [15]. In addition, preload has been shown to decrease after propofol [16, 17]. However, Carlier and others [18] and Lippmann and others [19] were unable to demonstrate a decrease in SVR after propofol. Thus there are at least 17 studies supporting a decrease in SVR after propofol and only two studies not confirming this.

It has been shown consistently also that cardiac output or variables reflecting it decrease after propofol [1-4, 7, 8, 10, 14, 16-18, 20, 21]. Thus SVR decreases and myocardial depression also occurs after propofol [21]. However, even patients with coronary artery disease seem to tolerate these effects well [21].

In the discussion in our paper published in *British Journal of Anaesthesia* [22], we erroneously included the paper by Lippmann and others [19] amongst those noting a decrease in SVR after propofol; we apologize for this mistake.

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PHARMACOKINETIC MODELLING OF MIVACURIUM

Sir,—Cook and colleagues [1] recently proposed an unconventional approach to estimating the pharmacokinetics of mivacurium, because of inability of their assay to distinguish the three stereoisomers of mivacurium, one of which (*cis-cis*) appears to have pharmacokinetics markedly different from those of the others [2] (and is believed to be only 10% as potent). When re-analysis of certain samples from the terminal phase using a stereospecific assay suggested that only the *cis-cis* isomer persisted, Cook's group assumed that the *cis-cis* isomer distributed to only one compartment and that its concentration throughout distribution and elimination could be estimated by extrapolating the log-linear terminal portion of the plasma concentration *vs* time curve to time zero (fig. 1). The difference between this curve and the total

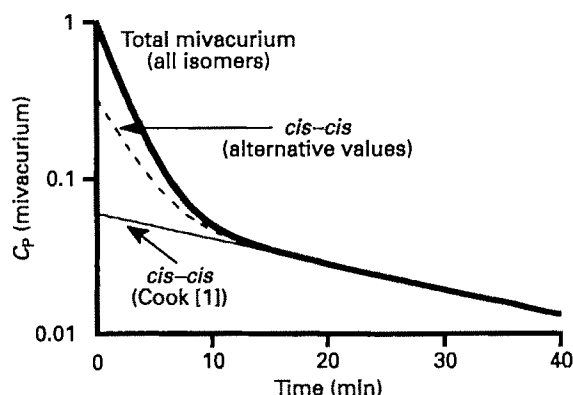


Fig. 1. The approach used by Cook and colleagues [1] to estimate the pharmacokinetics of mivacurium. C_p = Plasma concentration, normalized to 1 at time 0. — = Combined concentration of the three stereoisomers of mivacurium. — = Values assumed by Cook's group for the *cis-cis* isomer, assuming that this isomer distributes to only one body compartment and that the terminal portion represents only the *cis-cis* isomer. --- = An alternative concentration-time curve for the *cis-cis* isomer (one of many possible curves), assuming distribution into more than one compartment.

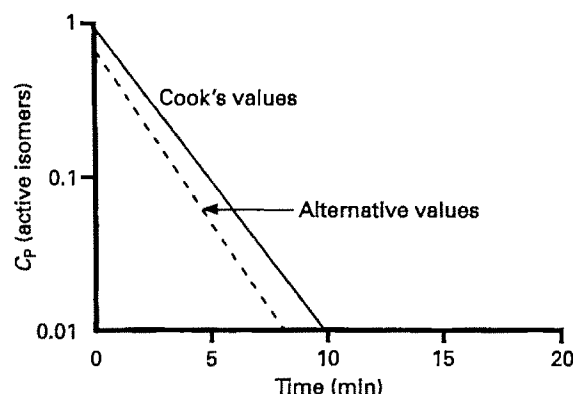


Fig. 2. Cook and colleagues' prediction [1] for the concentration of the active isomers (—; difference between Cook's total and proposed *cis-cis* concentrations (the thick and thin lines in figure 1)); --- (difference between the total and our proposed *cis-cis* concentrations) = an alternative estimate for these isomers. Our proposed values would result in markedly different estimates for clearance and volume of distribution than those reported by Cook's group.

mivacurium concentration (the sum of the three isomers) was assumed to represent the sum of the two "active" isomers (*trans-trans* and *cis-trans*). We disagree with the assumption by Cook and colleagues that the *cis-cis* isomer should distribute to only one compartment. Neuromuscular blockers, being polar, typically distribute to at least two compartments: central compartment volume is typically similar to that of plasma volume and volume of distribution at steady state generally approximates that of the extracellular fluid space. Mivacurium should distribute to similar body compartments.

Assuming that the isomers of mivacurium obey these principles, an alternate time course for the *cis-cis* isomer is likely; one possible curve for this isomer is shown in figure 1. The resulting values for the active isomers differ markedly from those proposed by Cook and colleagues [1] (fig. 2) and the true area under the curve (AUC) and area under the moment curve (AUMC) are likely to be smaller than Cook's group estimated. As a result, the reported estimates of clearance (calculated as dose divided by AUC) and volume of distribution (calculated using both AUC and AUMC) are probably incorrect.

Pharmacokinetic modelling of mivacurium may provide insight into the clinical effects of this novel neuromuscular blocker. However, correct kinetic analysis of mivacurium requires use of a stereospecific assay such as that recently reported by Lien and colleagues [2]. In addition, the elimination of mivacurium via cholinesterase may necessitate using non-traditional pharmacokinetic models such as those suggested by Hull for atracurium [3, 4]. We eagerly await these studies.

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Sir,—Our study was originally designed to examine the pharmacodynamics and pharmacokinetics of a single dose of mivacurium in normal patients and in those with hepatic or renal failure. We and others have performed similar studies with a variety of non-depolarizing neuromuscular blockers and other anaesthetic agents.

The pharmacokinetic data analysis in this mivacurium study was, indeed, complicated by our finding a terminal elimination half-life an order of magnitude greater than that predicted from either the increase in duration of neuromuscular block in response to doubling the mivacurium dose in our other clinical studies or the plasma hydrolysis rates for mivacurium which we have studied *in vitro*. This conflict was largely resolved conceptually when a new stereospecific assay for mivacurium was later developed and differences in the rates of metabolism of the three mivacurium isomers and their neuromuscular potency ascertained. These studies demonstrated that the *cis-cis* isomer of mivacurium represents some 4-8% of an injected dose, has limited potency compared with the active *cis-trans* and *trans-trans* isomers, and is metabolized slowly. Our study was thus seminal in calling attention to this interesting pharmacokinetic/dynamic problem and serving as the impetus for its resolution. The studies by Lien and colleagues provide a natural sequel to our study.

In an attempt to estimate the pharmacokinetics of mivacurium, we assumed that the terminal phase represents primarily the *cis-cis* isomer. Standard curve-stripping techniques were used to subtract the presumed contribution of the *cis-cis* isomer from each

plasma concentration-time data point. As stated clearly in our paper, one assumption of this method is that the *cis-cis* isomer obeys a one-compartment model and that the model does not account for the distribution of the *cis-cis* isomer. Thus we were quite aware that we had not accounted for a distribution phase for the *cis-cis* isomer and likewise quite aware of the principles of drug distribution. The purposes of pharmacokinetics are to facilitate calculations and to enhance our understanding of drug distribution. Our unconventional approach to modelling may introduce departure from mathematical "purity" and introduce some small computational error, but it certainly increased our understanding of the kinetics of mivacurium and provided a first estimate of its kinetic profile.

The distribution curve for the *cis-cis* isomer does have the shape proposed by Fisher and Hale in their figure 1. The area bounded by the *cis-cis* alternative values and the "*cis-cis* (Cook [1])" values (—) represents largely the potential error produced by our methods. We have estimated that this segment (or small triangle) produces a 3–5% error in our computations. In the past, we have shared most of our published and unpublished data from this study with Fisher and Hale; thus we are quite perplexed at the alternative time course for the *cis-cis* isomer which they now

propose. As the *cis-cis* isomer accounts for only 4–8% of the injected dose, one wonders how the time zero concentration (C_0) of the *cis-cis* isomer mivacurium could be 35% of the total isomers as depicted by Fisher and Hale in their figure 1. In our paper, we estimated the C_0 *cis-cis* mivacurium concentration to be 6.5 (SD 4.7)% of the estimated total mivacurium concentrations. This estimate seems much more realistic than that proposed by Fisher and Hale. Thus the distribution curve for the *cis-cis* isomers provided by Fisher and Hale seems of dubious relevance; the resulting value for the active isomers is probably equally flawed.

Various current investigations are studying the pharmacokinetics of mivacurium using stereospecific assays and various kinetic models. Such data will, indeed, provide further insights about the kinetics of this unique neuromuscular blocking drug. However, little insight and certainly no new information or new concepts are provided by drawing imaginary but unrealistic distribution curves for the *cis-cis* isomer.

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BOOK REVIEWS

Life Threatening Problems in the Emergency Room: Problems in Anaesthesia. Edited by A. Sutcliffe. Published (paperback) by Butterworth-Heinemann, Oxford. Pp. 252; indexed. Price £15.95.

This book is a pocket-sized paperback some 250 pages in length. The problems referred to in the title are dealt with in four main sections comprising trauma, burns, medical and surgical emergencies and, finally, paediatrics. Each section is subdivided logically into theoretical aspects, pathophysiology, practical aspects and management.

The text is a comprehensive and friendly description of the clinical problems which a junior anaesthetist may face in the resuscitation room of any Accident and Emergency department in the U.K. The author has based her presentation on personal experience and uses phrasing such as "most patients wake up quickly". She also suggests, for example, that the place to consider the theoretical aspects of triage for major trauma victims is "a leisurely bath". The presentation of what is a wealth of detail contains some unavoidable repetition and cross referencing, but this does not detract from the flow of information provided. Several useful tables appear, but there are no diagrams. References for further reading make frequent appearances.

Although the literary style will not be to everyone's taste, this is a very detailed book and contains much useful information for the novice, including, for example, just how tightly to bandage a chest. It is not an alternative to the ATLS and ACLS manuals, but rather a background description of the many problems which may present in a busy A & E department. The book should be used to provide basic knowledge rather than serving as an on-the-spot reference. It will complement the departmental library for both anaesthetists and surgeons working in A & E medicine.

D. Raitt

Cancer Pain. Edited by R. B. Patt. Published by J. B. Lippincott Company, Philadelphia. Pp. 650; indexed; illustrated. Price £79.50.

The management of pain is a vital aspect of the comprehensive care of a patient with cancer. The notion of cancer is inextricably linked with the image of pain and suffering and it is crucial, therefore, that pain assessment and management are integrated into cancer treatment. Unfortunately, in spite of changes in attitude and medical interest in this area, the WHO estimates that only 50% of cancer patients in developing countries receive effective pain relief. This truly excellent book is essential reading for those endeavouring to improve this situation.

The editor intentionally has adopted a broad approach in recognition of the input that various medical specialties have in the treatment of cancer pain. The book is intended as a comprehensive reference for those doctors who have a major commitment to cancer pain management and those only occasionally involved. It is well laid out, easy to read and generously

The section on pharmacological treatment discusses extensively all aspects of opioid, non-opioid and adjuvant drug usage and, provocatively, the reasons why medication is often not used to its advantage. The text is not cluttered by use of both generic and proprietary drug names, full guidelines on prescribing being well tabulated in Appendix C. I would disagree with the statement that the syringe driver is a "relatively unsafe" mode of delivery of s.c. opioids to cancer patients. It is used extensively in this country and has been shown to be both effective and safe.

Section three is entitled "Non-Pharmacological Treatment and Novel Approaches to Management". It contains useful information on the role of chemotherapy, radiotherapy and orthopaedic intervention. The chapter on intraspinal opioid therapy is outstanding. All the various options available for administration are discussed comprehensively. The text is full of practical advice regarding the use of this technique and there is an admirable section on trouble-shooting—invaluable to those not yet fully experienced in management of patients receiving long-term analgesia via this route. The salutary chapter on complications of invasive procedures contains fascinating photographs and accounts of those problems which may arise.

The last section, special problem area, completes an exceptional book. Many aspects of the fascinating account of problems in developing countries apply also to those in which more resources are available. The chapter on childhood cancer is an excellent review of the similar and different pain problems encountered in this vulnerable group.

Especially interesting in the appendix section are the guidelines to home patients who have implanted extradural catheters. Many pain specialists are using contrast media daily and the appendix on this topic was illuminating.

This is an encyclopaedic text on cancer pain. It should be considered essential reading for those clinicians, of whatever discipline, who wish to improve the plight of the cancer patient in pain.

B. J. Collett

A Practice of Anaesthesia for Infants and Children, 2nd Edn. By C. J. Coté, J. F. Ryan, I. D. Todres and N. G. Goudsouzian. Published by W. B. Saunders Company-Harcourt Brace Jovanovich Inc., Philadelphia. Pp. 544; indexed; illustrated. Price £50.00.

The first impression given by this second edition of this text is of an elegant presentation. It has been enlarged considerably in comparison with the first edition produced in 1986. The title appears somewhat strange at first, but is taken from the well known book by Wylie and Churchill-Davidson. The aims of this edition are the same as those of the first, with nearly half the book given over to detailed chapters on physiology and pharmacology.

The first chapter is an excellent example of sound common sense applied to the practice of paediatric anaesthesia. Later chapters suffer from the problem of multiple authorship, in that

circumstances and the continuing promotion of awake intubation of the neonate. The preference for inhalation induction reflects the unavailability of EMLA cream in the U.S.A. The use of extracorporeal membrane oxygenation in cases of diaphragmatic hernia continues to be questioned, in that patients with no chance of survival because of lung hypoplasia can be kept alive for considerable periods. Early extubation for patients with oesophageal atresia is not practised routinely on this side of the Atlantic. The use of metoclopramide in children is not recommended in the U.K. because of the high incidence of extrapyramidal effects. A personal foible of this reviewer is a strong dislike of the use of unrelated landmarks for venous cannulation.

Techniques recommended for the general anaesthetist should be transferable, if possible, from the adult. As one who has to anaesthetize the occasional obese adult female, the relationship of the nipple to the internal jugular vein escapes me!

The number of references is impressive, with only eight of the 29 chapters having fewer than 100 references; the chapter on anaesthetic pharmacology boasts 417. The majority of the references are well up to date.

Conversely, only one typographical error was noted, in the misspelling of coloboma. The criticisms are few, taking into account trans-Atlantic differences in practice. This must be one of the best textbooks of paediatric anaesthesia available. It can be recommended to anyone involved in the anaesthetic management of children.

D. Arthur

Anatomy for Anaesthetists, 6th Edn. By H. Ellis and S. Feldman. Published by Blackwell Scientific Publications Ltd. Pp. 358; indexed; illustrated. Price £49.50.

It has been 5 years since the previous edition of this textbook. This most recent edition has a larger page size and there have been some additions to take account of developments in anaesthetic practice. The book is divided into eight parts covering all the major aspects of human anatomy. Special zones of anaesthetic interest are described in the final section. These are very useful for the candidate preparing for the postgraduate examinations in anaesthesia.

A section has been added on the airway during anaesthesia and the contribution of the soft palate to airway obstruction is discussed with references to some of the recent work in this area.

The use of the laryngeal mask has expanded rapidly since 1988,

and this device has a brief mention. In a book that is notable for the accuracy of its diagrams and drawings, I was disappointed that the diagram of the laryngeal mask *in situ* is inaccurate as it shows the mask pointing in a posterior direction when, as may be seen in the accompanying lateral x-ray, the laryngeal mask usually faces anteriorly when positioned correctly.

The Mallampati classification of the airway is outlined in the paragraph on difficulties in tracheal intubation.

The text of this book has been revised in keeping with present day nomenclature and, as in previous editions, the book is illustrated by many clear diagrams and drawings.

Anaesthetists in training who are studying for the F.R.C.A. will find this book a valuable aid, as have many anaesthetists over the past 30 years since the first edition.

J. A. Langton

MCQ Tutor in Basic Sciences for Anaesthesia. By C. A. Pincock and R. P. Jones. Published by Churchill Livingstone, Edinburgh. Pp. 150; indexed. Price £11.50.

This book provides a collection of 240 MCQ in basic sciences (120 each in pharmacology and physiology) directed at candidates preparing for the Part 2 F.R.C.A. examination. The book describes the structure of the examination and gives advice on MCQ technique. There are six sets of 40 questions with alternate sets on pharmacology and physiology.

The authors have proposed that this book should be used as a test of knowledge and as a guide to learning. To this end, the format differs from some other collections of MCQ, in that each question is preceded by a rather wordy preamble outlining what basic knowledge is required in the subject of the question and what closely related topics may occur. The answers are backed up with an explanation and a source reference provided. The references are a useful contribution to the learning process. I am less sure about the preamble to each question; it may help the candidate to think along the right lines in answering the question, but it makes it difficult to use as a "mock" examination. In this respect, the layout of having the answers on the page opposite to the questions makes it difficult to use, although the authors suggest covering the opposite page while attempting the question.

Overall, I think this is a useful book for Part 2 candidates which provides an appropriate range of questions.

C. S. Reilly

ERRATA

British Journal of Anaesthesia 1991; 67: 539–545

The co-ordinate labelled $ED_{50}P$ in figure 4 (p. 543) should read $ED_{50}P$.

British Journal of Anaesthesia 1993; 70: 562–573

p. 567: *Fluids and electrolytes*. The first sentence should read:

Ensure intravascular volume repletion with colloid, then give 1 litre of saline solution, either 150 or 30 mmol litre⁻¹, every 4–6 h.

In the same section, two subsequent references to "saline 0.3 mol litre⁻¹" should read "saline 30 mmol litre⁻¹".

We apologize to the authors for this confusion, which arose during the editorial process.

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EDITORIAL

PREDICTING DIFFICULT INTUBATION

Research aimed at predicting difficult tracheal intubation falls into two categories. One is concerned with defining the underlying anatomical problems without necessarily producing a useful clinical test, the other is aimed directly at the clinical need for a simple bedside test that will predict difficult intubation.

As difficult *intubation* occurs infrequently and is not easy to define, research has been directed at predicting difficult *laryngoscopy*. This is graded as the amount of larynx seen when a Macintosh laryngoscope is used in a patient with the head and neck in the optimum position. Most research workers have defined "difficult laryngoscopy" as failure to see beyond the epiglottis. It is argued that, if difficult laryngoscopy has been predicted and intubation is essential, skilled assistance and special equipment can be provided.

The pioneer work in the first category of research was carried out by two ENT surgeons, White and Kander [1] using head and neck x-rays. More recently, this work has been repeated, extended and subjected to appropriate statistical analysis by Bellhouse and Doré [2]. Horton, Fahy and Charters [3] have obtained lateral x-rays of the head and neck during laryngoscopy to show the relationship of the laryngoscope blade to the hyoid, and Bucx and colleagues [4] have measured the forces applied during laryngoscopy, relating them to patient factors. The intention of this type of research is to reveal differences that may lead to the design of better clinical predictors of difficulty or improvements in current methods of laryngoscopy. In this issue of the journal, Chou and Wu present x-ray evidence from a small number of patients known to present difficulty in laryngoscopy, that the vertical distance between the mandible and the hyoid was substantially longer than in a control group [5]. This is confusing as, in clinical studies, others have worried about a high, or "anterior" larynx [6] and Pottecher and colleagues [7] found the mento-hyoid distance to be shorter (but not usefully so) in patients who presented difficulty in laryngoscopy or intubation.

The second category of research is aimed directly at producing a simple bedside test that will reliably predict those patients in whom laryngoscopy may be difficult. The test most widely advocated is that devised by Mallampati and colleagues [8]. Its great merit is simplicity, but those who recommend it completely ignore its unreliability. The principle is simple enough—when the mouth is opened and the tongue protruded, a large or disadvantageously positioned tongue obscures the view of the posterior

pharynx and therefore may also obscure the view of the glottis. As many as four grades of pharyngeal view have been described [9], but in practice only the boundary between two is used as the criterion for predicting difficult laryngoscopy. The exact definitions vary slightly between authors, adding to the confusion. The clearest and simplest version is: if you cannot see any of the posterior pharyngeal wall, predict "difficult" [10]. Regrettably, the test performs poorly, and about 50% of the patients who proved to be difficult to laryngoscope were not predicted as such [7, 8, 11–14]. This is no great surprise, as the test has considerable observer variability [11, 15, 16], and does not take head and neck movement into account.

The test designed by Wilson and his colleagues [17] combines into a predictive rule several factors found to be associated with difficult intubation. This test is quick, far simpler to use than might at first appear from the description, and has the advantage of focusing attention on individual factors. When it was used by eight anaesthetists (seven of whom had never used it before), 80% of patients in whom laryngoscopy subsequently proved difficult were identified correctly. Unfortunately, Oates and colleagues [11] reported a much worse performance in a comparative study, finding little to choose between the Mallampati and Wilson tests, with both tests failing to predict as many as 58% of difficult laryngoscopies.

Combinations of various measurements, including some new ones, are being examined in the hope of improving predictive ability. There is no advantage in combining the Wilson and Mallampati tests [18]. A deficiency of many tests is their failure to give due weight to movement at the atlanto-occipital joint [6] and more especially the atlanto-axial gap [19], which is now known to be especially important. Menon [cited in 20] has suggested it can be assessed better by asking the patient to extend the head while their neck is held in full flexion. The thyro-mental distance is potentially useful because it is easy to measure and is influenced by several factors. As a single test it is unreliable [13], but it may work if combined with other tests. Frerk [10] has argued that, if the thyro-mental distance is less than 7 cm and the posterior pharyngeal wall cannot be seen during the Mallampati test, intubation will be difficult. Unfortunately, the majority of difficult intubations thus identified would not have met the criterion for difficult laryngoscopy used by other authors [20], so the case remains unproven. Two recent studies published in abstract, one of 1501 patients [21] and

the other enriched with patients known to be difficult to laryngoscope [22], failed to predict difficult laryngoscopy reliably, despite examining many potential predictors.

The search for improved predictive tests continues, but is beset with difficulties, not the least of which is the decline in the number of tracheal intubations and, consequently, a decline in the number of "difficult patients" studied. As difficult laryngoscopy is a multifactorial problem, many such patients must be studied to ensure the necessary spread of factors. Even quite large studies usually contain a number of suitable patients insufficient to provide acceptable estimates (95% confidence limits) of predictive ability. So, despite claims, there is as yet insufficient evidence to choose between the Mallampati, Wilson and Frerk tests, or any other combination. Finally, any new predictive rule must be tested prospectively on a large sample of new patients, as a test inherently performs well on the data used to create it. This is an important point of experimental design that is usually forgotten or ignored.

Meanwhile, also, anaesthetic techniques have been changing. Fewer patients undergo tracheal intubation, because of the increasing use of the laryngeal mask and regional anaesthetic techniques (especially in obstetrics), and so fewer patients are being put at risk. Fibreoptic intubation is practised widely and other devices have some advocates. More important, the gum elastic bougie is used widely in the U.K. for difficult intubation. The success of this simple tool has quietly but radically altered anaesthetic practice, as all that is now required is a view of the epiglottis: even when the cords cannot be seen, the tip of the gum elastic bougie can be slipped quickly behind the epiglottis, through the cords, and a tracheal tube railroaded over it into position [23]. Another very promising device described recently by McCoy and Mirakhur [24] is a levering laryngoscope that lifts the tip of the epiglottis out of the line of vision. Consequently, the requirement for a test has shifted to a different goal—the prediction of *failure to see the epiglottis*. This is quite a rare event (and consequently even more difficult to study with any statistical confidence).

No test is likely to be perfect. Therefore, it remains essential that every anaesthetist must be trained and equipped to deal with the now much less common, unexpected failure to intubate. This is more important than reliance on a fallible test.

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Bath

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ORIGINAL ARTICLES

MANDIBULOHYOID DISTANCE IN DIFFICULT LARYNGOSCOPY

H.-C. CHOU AND T.-L. WU

SUMMARY

We studied radiographically 11 patients in whom direct laryngoscopy proved difficult and 100 control (general population) subjects. The vertical distance between the mandible and the hyoid bone (mandibulohyoid distance) was measured and the positions of the mandibular angle and hyoid bone determined in relation to the cervical vertebrae. We found that the mandibulohyoid distance was substantially longer in patients whose trachea was difficult to intubate; the mandibular angle tended to be positioned more rostrally in both men and women, and the hyoid bone tended to be positioned more caudally in women. This suggests that a relatively short mandibular ramus or a relatively caudal larynx may be important, unfavourable anatomic factors in difficult laryngoscopy. (Br. J. Anaesth. 1993; 71: 335–339)

KEY WORDS

Airway: anatomy, tongue. Intubation, tracheal: laryngoscopy.

Although difficult intubation continues to cause morbidity and mortality associated with anaesthesia, the reasons for difficult laryngoscopy have not been completely identified or explained. In the past, difficult intubation was attributed to several unfavourable anatomical factors such as receding mandible, protruding upper incisors and long maxilla, limited mobility of the temporomandibular joint [1, 2], small atlanto-occipital gap [3, 4], restricted pharyngeal space, and reduced submandibular tissue compliance [5]. Difficult laryngoscopy apparently is associated with one or more of these factors. Our study was designed to investigate the aetiology of difficult laryngoscopy in 11 patients with documented problems and to define a possible predictive indicator, mandibulohyoid distance.

SUBJECTS AND METHODS

We obtained lateral cervical radiographs of 11 patients in whom intubation during routine surgical procedures was found to be difficult (conventional rigid laryngoscopy by experienced anaesthetists failed). For clinical comparison, we also included one radiographic study of a patient with a severely receding jaw and protruding upper incisors ("buck" teeth), in whom laryngoscopy was anticipated as difficult before anaesthesia but was performed without difficulty.

The lateral cervical x-rays were taken with the head in the neutral position and the mouth closed. The atlanto-occipital gap was measured from the upper margin of the posterior tubercle of atlas vertically upward to the occiput. The rostro-caudal positions of the mandibular angle and hyoid bone were determined by horizontal extension to the corresponding levels of the cervical vertebrae (fig. 1). The mandibulohyoid distance, defined as the vertical distance between the mandible and the hyoid bone,

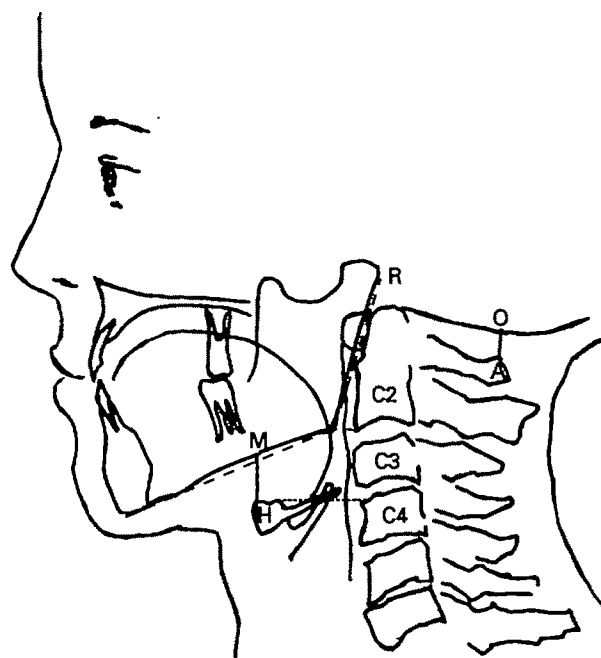


FIG. 1. Diagram of normal lateral cervical x-ray film of female patient with head in neutral position, to show how radiological results were obtained. Atlanto-occipital gap was measured from upper margin of posterior tubercle of atlas (A) vertically upward to occiput (O). Mandibular angle was determined by drawing a horizontal (—) line from the intersection of two tangents of posterior ramus (R) and lower border of the mandible (M), across to the cervical (C2) spine. The position of the hyoid bone (H) was determined by drawing a horizontal line (----) from the upper margin of the hyoid bone to the adjacent cervical spine. The mandibulohyoid (MH) distance was measured from the upper margin of the hyoid bone (H) vertically upward to the lower margin of the mandible (M). Note that mandibular angle is situated at the lower C2 level, and H is situated between C3 and C4.

This article is accompanied by an Editorial.

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TABLE I. Radiological findings. AOG = Atlanto-occipital gap; MH = mandibulohyoid. Unfavourable anatomical factors: 1 = small atlanto-occipital gap; 2 = rostral mandibular angle (short mandibular ramus); 3 = caudal hyoid bone (caudal larynx). * Patient in whom intubation was anticipated as difficult but was performed without difficulty

Patient No.	Sex	Age (yr)	AOG (mm)	Cervical vertebra nearest		MH distance (mm)	Physical characteristic	Unfavourable factors
				Mandibular angle	Hyoid bone			
1	M	68	0	Upper C2	C3 to C4	45	Severely receding jaw	1, 2
2	M	63	5	Middle C2	Upper C4	31	Receding jaw	2
3	M	36	7	Middle C2	Lower C3	32	Bull neck	2
4	M	50	7	Middle C2	C3 to C4	38	None	2
5	M	38	2	Lower C2	C3 to C4	20	None	1
6	M	63	0	C2 to C3	Lower C4	37	Very short neck	1, 3
7	F	48	4	Middle C2	Middle C4	30	Receding jaw	2, 3
8	F	56	2	C1 to C2	Middle C3	22	Receding jaw	1, 2
9	F	44	4	Lower C2	Middle C4	30	Slightly receding jaw	3
10	F	47	0	Middle C2	C2 to C3	16	None	1, 2
11	F	53	5	Middle C2	Middle C4	34	None	2, 3
12*	F	41	7	Lower C2	Lower C3	14	Severely receding jaw, buck teeth	None

was measured from the upper margin of the hyoid bone vertically upward to the lower margin of the mandible. Radiological findings are summarized in table I. Because none of our subjects had a prominent long maxilla or temporomandibular joint problems, we did not include assessment of these two factors in our study.

In order to select a control group, we examined cervical spine x-ray films (taken by the radiology department during a 2-month period) in which the patient's head was in the neutral position; we excluded films of paediatric and edentulous patients. We measured the mandibulohyoid distance and determined the positions of the mandibular angle and the hyoid bone in 100 patients (54 male).

All data collected from our control and study subjects were analysed using appropriate statistical tests.

RESULTS

Difference in mean mandibulohyoid distance between the study and control groups

Data were analysed separately by gender, using a two-sided *t* test; the level of significance was $\alpha = 0.05$. The difference between mean mandibulohyoid distances in male study subjects (mean 33.8 (SD 8.4) mm) and male control subjects (mean 21.4 (8.6) mm) was significant ($P = 0.0013$), as was that between female study subjects (26.4 (7.3) mm) and female control subjects (15.4 (6.3) mm) ($P = 0.0006$); this reflected a wide variation in mandibulohyoid distances in the general adult population. Patients in whom the trachea was difficult to intubate, however, tended to have a relatively longer mandibulohyoid distance (fig. 2).

Association of mandibulohyoid distance with vertebral levels of mandibular angle and hyoid bone (table II)

Linear regression models including as covariates vertebral levels for both the mandibular angle and hyoid bone were used to test statistical significance of associations with mandibulohyoid distance. In women, both relatively "rostral" mandibular angle (middle C2 and above) and relatively "caudal"

hyoid bone (middle C4 and below) were associated with longer distance; the association was stronger for the hyoid bone ($P = 0.0001$) than for the mandibular angle ($P = 0.0121$). In men, longer distance was associated with relatively rostral mandibular angle ($P = 0.0003$), but not with the position of the hyoid bone. Therefore, although the mandibular angle was generally situated at the lower C2 level and the hyoid bone between the C3 and C4 levels (fig. 3), in

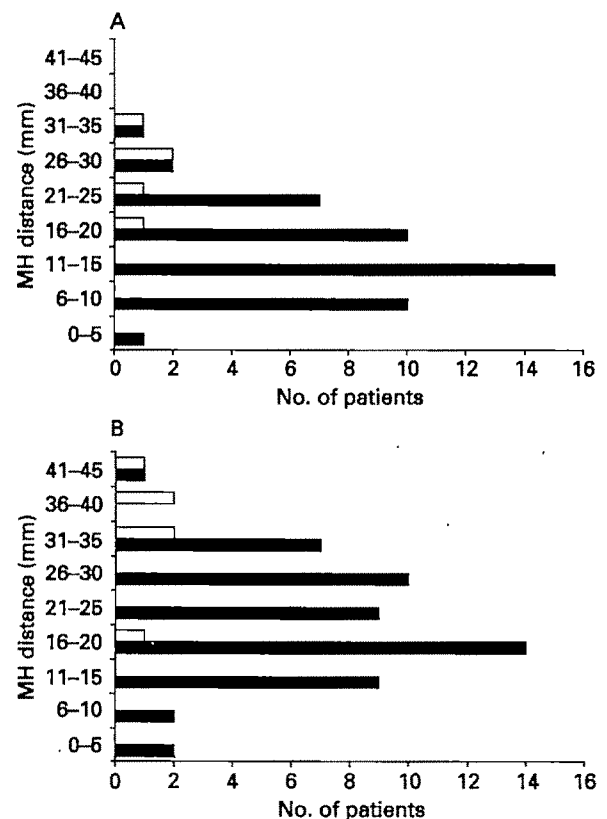


Fig. 2. Mandibulohyoid (MH) distance in study (□) and control (■) subjects. A: Women ($n = 5, 46$; mean 26.4, 15.4 mm; range 16-34, 1-34 mm, in study and control subjects, respectively); B: men ($n = 6, 54$; mean 33.8, 21.4 mm; range 20-45, 2-42 mm, in study and control subjects, respectively). MH distance varied widely in control subjects, but study subjects tended to have a relatively long MH distance. Difference between mean MH distance in study and control subjects significant for both males and females.

TABLE II. Mean (SD) mandibulohyoid distance (MHD) by vertebral levels of the mandibular angle and hyoid bone for the study (n = 11) and control groups (n = 100) combined

Vertebral level	Men (n = 60)		Women (n = 51)	
	No.	MHD (mm)	No.	MHD (mm)
Mandibular angle				
Middle C2 and above	7	34.4 (6.2)	11	22.2 (8.1)
Lower C2, C2 to C3	41	23.1 (7.6)	34	14.9 (5.9)
Upper C3 and below	12	14.7 (9.4)	6	15.3 (7.2)
Hyoid bone				
Lower C3 and above	13	22.5 (7.0)	23	14.1 (5.4)
C3 to C4, upper C4	38	22.9 (9.4)	22	15.7 (5.4)
Middle C4 and below	9	22.6 (13.4)	6	28.8 (6.3)

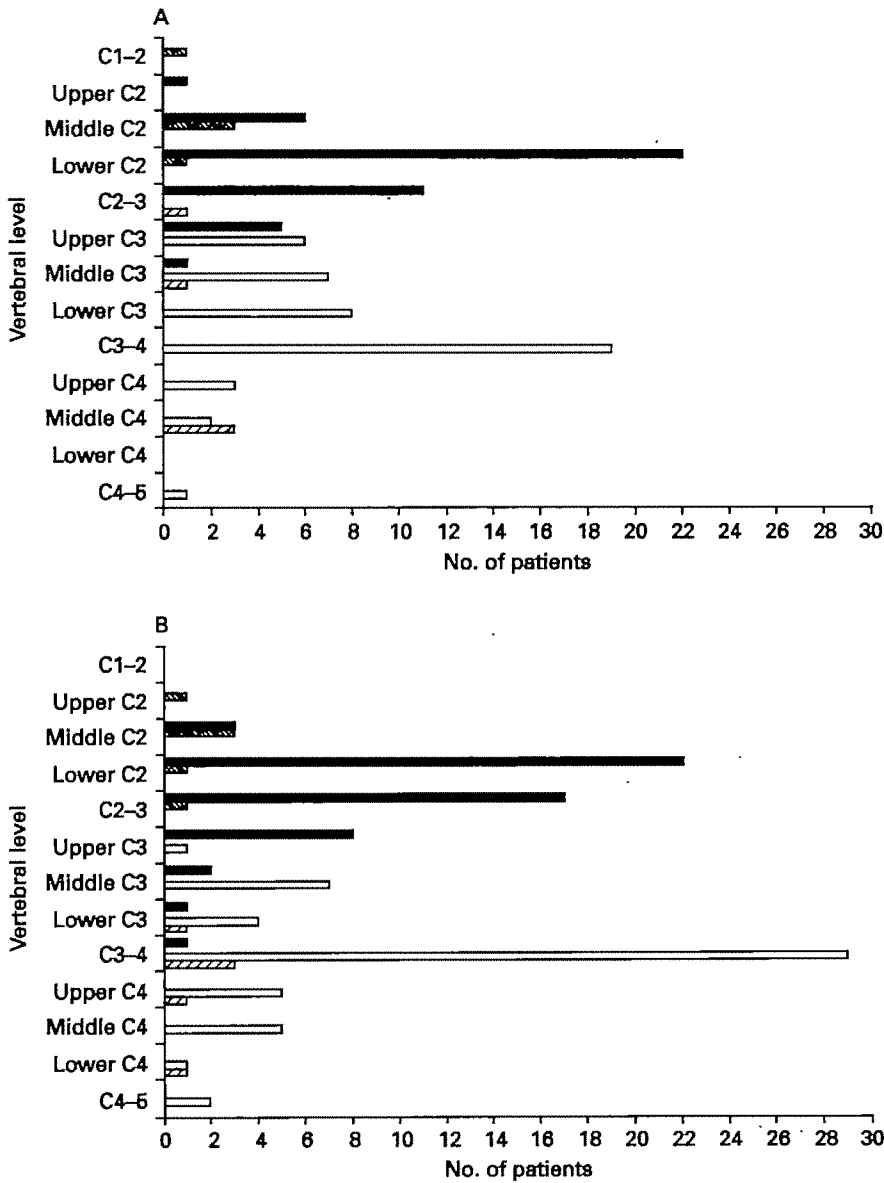


FIG. 3. Vertebral level of mandibular angle (■, ▨) and hyoid bone (□, ▤) in control and study subjects, respectively. A: Women (control n = 46; study n = 5); B: men (control n = 54; study n = 6). The mandibular angle was generally situated at the lower C2 level and the hyoid bone was generally situated between C3 and C4 levels in control subjects. Study subjects had a relatively rostral mandibular angle (both men and women) and a relatively caudal hyoid bone (women only).

patients with relatively longer mandibulohyoid distance the mandibular angle tended to be situated more rostrally among both men and women, and the hyoid bone tended to be situated more caudally among women.

Distributions of mandibular angle and hyoid bone position

The proportion with relatively rostral mandibular angle and relatively caudal hyoid bone for the study



FIG. 4. Lateral cervical x-ray film of patient No. 1 (male) shows unusually long mandibulohyoid distance (45 mm), unusually short mandibular ramus (mandibular angle is situated at upper C2 level) and occiput in contact with atlas in neutral position (atlanto-occipital gap = 0 mm). Note that a large portion of tongue mass is situated in the hypopharynx.



FIG. 5. Lateral cervical x-ray film of patient No. 12 (female) shows buck teeth and receding jaw. Mandibulohyoid distance (14 mm), mandibular angle (at lower C2 level) and hyoid bone (at lower C3 level) are relatively normal. Intubation was not difficult.

group was compared with that of control subjects using Fisher's exact test. The study group had a larger proportion with rostral mandibular angle in both men ($P = 0.0001$) and women ($P = 0.0006$). The proportion with a relatively caudal hyoid bone was larger for the study group in women ($P = 0.0009$), but not in men. This analysis suggested further that patients who presented difficulty in intubation, tended to have a relatively rostral mandibular angle; although a relatively caudal hyoid bone was also found significant for women in this group.

DISCUSSION

Because it measures the vertical distance between the mandible and the hyoid bone, the mandibulohyoid distance also indicates the relative rostro-caudal positions of the two. A more rostral mandibular angle, a more caudal hyoid bone, or both, contribute to a longer mandibulohyoid distance.

The ramus of the mandible articulates rostrally with the base of the skull at the mandibular fossa and extends caudally into the cervical region. A more rostral mandibular angle thus indicates a relatively short mandibular ramus. Because the epiglottis arises from the thyroid cartilage and then remains dorsal to the hyoid bone, the position of that bone marks the entrance to the larynx. A more caudal hyoid bone thus indicates a relatively caudal larynx.

Role of a long mandibulohyoid distance in difficult laryngoscopy

In the neutral head position, the oropharyngolaryngeal passage has two axes which form an angle of almost 90° [6]. During normal laryngoscopy, we first convert that angle to about 125° by extending the head at the atlanto-occipital joint, and then use the laryngoscope blade to displace the tongue and establish a 180° straight-line alignment.

Basic geometric principles show that a small atlanto-occipital gap, protruding upper incisors and a long maxilla, a caudal larynx, or a combination of any of these anatomical factors, necessitate a greater degree of tongue displacement. If the submandibular tissue compliance is sufficient to compensate for these unfavourable factors, tongue displacement is not difficult; otherwise, difficulty may be anticipated.

A short mandibular ramus raises the floor of the oral cavity toward the base of the skull. Minor reduction in the linear vertical length of the mandibular ramus may substantially reduce the size of the oropharyngeal cavity, diminishing the space available for tongue displacement and thus contributing greatly to overall reduction of submandibular tissue compliance during laryngoscopy. When the position of the hyoid bone is caudal or is relatively caudal because of a short ramus, a large portion of the tongue is situated in the hypopharynx instead of in the oral cavity (fig. 4). During laryngoscopy, this large hypopharyngeal tongue mass further compromises the compliance needed for its displacement. The effects of a short mandibular ramus and a caudal hyoid bone explain the difficult intubation in patients Nos 4 and 11, who had no other indicators of a difficult laryngoscopy.

Comparative observations

Thyromental distance and horizontal length of the mandible are often used to assess the mandibular space and serve as a predictor for difficult laryngoscopy [7]. However, when this assessment was applied to patients Nos 1 and 12, both of whom had a severely receding jaw, it contradicted the clinical findings: in patient No. 1, who had an unusually long mandibulohyoid distance (45 mm), thyromental distance was 7.5 cm (i.e. > 6 cm) and horizontal length of the mandible was 7.5 cm (i.e. < 9 cm); in patient No. 12 (fig. 5), who had a relatively normal mandibulohyoid distance (14 mm), thyromental distance was 5.5 cm (i.e. < 6 cm) and horizontal length of the mandible was 7.8 cm (i.e. < 9 cm), but intubation was not difficult.

These findings may be explained as follows. First, thyromental distance, which is measured obliquely, is greatly affected by the vertical mandibulohyoid distance; patients in whom the mandibulohyoid distance is long tend also to have a long thyromental distance (patient No. 1).

Second, the micrognathic mandible, which may be deficient in width and length in both the body and the ramus, manifests clinically as a receding jaw, a short ramus, or both [8]. Although micrognathic patients often present difficulty in intubation, a short ramus may be a more important contributing factor than a receding jaw (for example, in patient No. 12, who had a relatively normal ramus, intubation was not difficult in spite of a severely receding jaw).

Unlike a receding jaw, a short mandibular ramus is a more subtle physical feature, and difficult intubation is often not suspected before anaesthesia (as in patient No. 4), although such a patient may be perceived as having low-set ears and a long neck.

An increased distance between the mandible and hyoid bone was referred to by Riley and colleagues [9] as "long MP-H", indicating a possibly inferiorly positioned hyoid bone. These authors described its role in cephalometric analysis for evaluating the obstructive sleep apnoea syndrome. Although the measurement was made differently from ours, it probably signified the presence of a similar anatomical deficiency (short mandibular ramus, caudal hyoid bone, or both), which results in difficult intubation.

In summary, difficult laryngoscopy is caused by complex dynamic interaction of many anatomical factors which aggravate or compensate for each other. If a favourable anatomical factor (such as an edentulous condition) exists, even an unusually long mandibulohyoid distance may not result in difficult laryngoscopy. However, a normal ramus or hyoid bone does not preclude difficult intubation if other unfavourable anatomical features are present (as in patient No. 5).

Preoperative examination is needed to quantify the combined effects of all unfavourable factors, including the short mandibular ramus and caudal larynx we have described. The mandible and hyoid bone are easily identified anatomical landmarks at radiological or physical examination. A long MH distance may be an additional clinical indicator for anticipating difficult intubation.

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PERIOPERATIVE MONITORING WITH PULSE OXIMETRY AND LATE POSTOPERATIVE COGNITIVE DYSFUNCTION

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SUMMARY

In a randomized, blinded clinical study, we have used objective and subjective measures to determine if perioperative monitoring with pulse oximetry—by virtue of its potential to lessen hypoxaemia—would decrease late postoperative cognitive dysfunction. We investigated 736 adult patients undergoing elective procedures (other than cardiac, neurosurgical or for cancer) under regional or general anaesthesia, allocated randomly to undergo (group I) or not to undergo (group II) pulse oximetry monitoring in the operating theatre and recovery room. Cognitive function was evaluated using the Wechsler memory scale (WMS) and continuous reaction time (RT) test the day before surgery, and on the 7th day after operation or at discharge if that occurred before postoperative day 7. A questionnaire sent 6 weeks after surgery elicited patients' subjective perceptions regarding cognitive abilities. There were no significant differences between the two groups in either the total WMS score, the score for each WMS subtests or RT test. The questionnaire revealed that 7% in group I and 11% in group II believed cognitive abilities had decreased (ns). For the 40 patients whose WMS scores were 10 points less after than before operation, a follow-up study was undertaken 3 months after surgery. At that time, the median WMS score had returned to the preoperative value. We conclude that, for these 736 patients, subjective and objective measures did not indicate less postoperative cognitive impairment after perioperative monitoring with pulse oximetry. (Br. J. Anaesth. 1993; 71: 340–347)

KEY WORDS

Complications: hypoxaemia. Memory.

Postoperative cognitive dysfunction has been reported even after non-cardiac and non-neurosurgical operations and anaesthesia [1–5]. Perioperative hypoxaemia has been suggested as a major risk factor leading to postoperative cognitive dysfunction [3, 6–10]. Impaired cognitive functions range from momentary postoperative confusion and delirium [8–10] to permanent brain damage [3, 6, 7]. In a few studies, postoperative patients were examined with neuropsychological tests [9, 10] and the role of

hypoxaemia was considered, as mild to moderate hypoxaemia is associated with cognitive dysfunction [11–14]. Recent studies have suggested that hypoxaemia in the operating theatre and recovery room is common [15–17], and that monitoring with pulse oximetry would make possible early diagnosis and treatment and thus reduce markedly the incidence and severity of this condition [18–20]. We postulated that perioperative monitoring with pulse oximetry should reduce the incidence or severity of perioperative cognitive dysfunction. In our recent, randomized evaluation of pulse oximetry in 20802 patients, we found no association between the rate of postoperative neurological complications and the use of pulse oximetry [21, 22]. However, because we searched only for protracted confusion, stroke and coma, more subtle and diffuse cognitive effects would have been missed. In this randomized study using objective and subjective measures, we have assessed any possible relationship between the use of pulse oximetry and late postoperative cognitive dysfunction.

PATIENTS AND METHODS

Because pulse oximetry was not widely used in Denmark at the time of the study, a controlled, randomized trial seemed to be an appropriate step in assessing the effect of this monitoring technique on anaesthesia care. The study was approved by the Regional Ethics Committee for Copenhagen and Esbjerg, and patients gave informed consent. The study was carried out during 15 consecutive months at two major Danish hospitals, one of which was a University hospital.

The patients were 18 yr or older, admitted for elective surgery requiring general, spinal or extradural anaesthesia expected to last more than 20 min. We excluded outpatients and patients undergoing

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neurological, cardiac or cancer operations. Patients with suspected or confirmed cancer were excluded because a pilot study of 100 subjects indicated a confounding psychological difference between patients with cancer and those without cancer [Moller JT, unpublished data, 1988]. The need for reoperation was another exclusion criterion.

Patients were allocated to an operating theatre the day before surgery according to the department's routine, after which time pulse oximeters were allocated randomly to 50% of those theatres. A sealed envelope contained the daily random assignment and was not opened until the patients had been allocated to an operating theatre. The random assignment of an individual patient to receive monitoring with pulse oximetry (group I) or not (group II) could not be changed by moving the patient to another OT. Every day, as many as four patients from each surgical department were chosen randomly to undergo cognitive testing. The patients selected for study alternated between the surgical specialties according to a predetermined rotation schedule known only by the investigators. The physicians allocating patients to the operating theatres did not know which patients would undergo cognitive testing.

Cognitive testing

Cognitive testing consisted of administration of the Wechsler memory scale (WMS) [23] and continuous reaction time (RT) test the day before operation (WMS I and RT I) and, after operation, on the 7th postoperative day or on the day of discharge from the hospital if that occurred before postoperative day 7 (WMS II and RT II). The tests were administered by specially trained nurses who were unaware of the random allocation of pulse oximetry monitoring. The WMS requires approximately 45 min to administer, and consists of a series of eight subtests of attention and memory, including personal and current information, orientation, mental control, logical memory, memory span, visual reproduction and associated learning [23]. It is available in two versions; both are standardized and contain the same number and kinds of subtests. As a check on reliability of the standardization, we used the two versions in random order for each patient. The WMS is perhaps the most widely used and acknowledged test of memory function.

The RT test requires approximately 8 min to complete and was administered with a computer (Olivetti M24) with specially designed software (developed by B. Fries, medical technician, T. Kann, medical engineer, I. Svernild, Ph.D., psychologist,

and J. T. Moller, M.D.). Patients were asked to respond to a series of 100 light flashes by pushing a button as quickly as possible with the dominant hand. The flashes occurred without warning every 1–8 s. A learning session using 25 light flashes familiarized the patient with the procedure before actual testing.

Patients were asked also to evaluate subjectively if their cognitive abilities had changed since surgery: a short questionnaire sent by mail 6 weeks after surgery elicited details of mood, memory and the ability to concentrate.

To study the influence of time on cognitive dysfunction following anaesthesia, we also studied a subsample of patients. All patients whose WMS score decreased by 10 points or more (total WMS score 93; median approximately 63) after operation were asked to participate in a new test 3 months later ("cases"). For each of these patients, a control patient was selected randomly from all study patients (monitored and not-monitored), without regard for WMS scores. For each patient, the version of WMS used before operation was used for the 3-month follow-up.

Monitoring and anaesthesia

In group I, pulse oximetry was begun just before induction of anaesthesia and continued until discharge of the patient from the recovery room. Two different pulse oximeters were used: an Ohmeda 3700 at one hospital and a Radiometer OXI at the other. A finger probe was used in all instances. All personnel were instructed to maintain oxyhaemoglobin saturation (Sp_{O_2}) equal or greater than 93% in group I. Any reading less than this required intervention. Exceptions to this policy could be sanctioned only by the anaesthetist in charge of the individual patient. In group II, patients were not monitored by pulse oximetry at any time. Other operating theatre monitoring consisted of continuous electrocardiography, measurement of arterial pressure at least every 5 min and, when warranted by the type of surgery or the patient's physical condition, measurement of central venous pressure. In the recovery room, arterial pressure, heart rate and ventilatory frequency were measured at least every 15 min; when indicated, electrocardiogram and central venous pressure were monitored continuously.

The preoperative visit, premedication, anaesthetic regimen, postoperative observation and other anaesthesia- and recovery-related care followed the routine guidelines of each hospital. Each patient's physician, unaware of the patient's possible allocation to oximetry monitoring, planned the

TABLE I. Patient data and duration of surgery. Group I: Monitored with pulse oximeter; group II: no pulse oximeter monitoring. *Significantly uneven distribution ($P < 0.05$)

	Group I (n = 358)			Group II (n = 378)		
	Mean	Median	Range	Mean	Median	Range
Age (yr)	52.2	56	18–81	52.3	55	18–82
Weight (kg)	74.2	73*	40–136	71.6	71*	45–115
Height (cm)	169.6	168	147–193	169.4	169	135–192
Duration of surgery (min)	88.0	90	20–320	89.0	88	20–430

TABLE II. Major patient-related risk factors, type of anaesthesia and surgical procedures. Group I: Monitored with pulse oximeter; group II: no pulse oximeter monitoring. No significant difference between groups

	Group I (n = 358)		Group II (n = 378)	
	No.	%	No.	%
Risk factors				
ASA				
I	223	62.3	249	65.9
II	111	31.0	110	29.1
III	23	6.4	19	5.0
IV	1	0.3	0	0.0
Heart disease	33	9.5	24	6.5
Lung disease	35	9.9	28	7.5
Anaesthesia				
General anaesthesia				
Potent inhalation	98	27.3	103	27.3
I.v.	83	23.1	86	22.8
Regional	163	45.5	172	45.5
GA+regional	14	3.9	17	4.5
Surgical procedure				
Eye and ENT	26	7.3	33	8.7
Upper abdominal	15	4.2	16	4.2
Lower abdominal	129	36.0	128	33.9
Procedures on extremities	172	48.0	186	49.2
Other procedures	15	4.2	16	4.2

anaesthetic regimen as usual. For premedication, patients received either a benzodiazepine orally or an opioid i.m. before induction of anaesthesia. General anaesthesia was classified as either inhalation or i.v., based on the primary maintenance agent used. The lungs of all patients were ventilated with an inspired oxygen concentration of at least 30 %. When applicable, residual neuromuscular block was antagonized with neostigmine 2.5 mg and atropine 1 mg i.v. Regional anaesthesia was either extradural or spinal block. Supplementary oxygen at a minimum flow of 3 litre min⁻¹ was administered to all patients during regional anaesthesia and to all patients after regional and general anaesthesia upon admission to the recovery room, where analgesia consisted of i.v., i.m. or extradural administration of morphine.

Data collection and analysis

Because information is limited on cognitive dysfunction beyond 4 days after routine anaesthesia, it was difficult to determine the precise number of patients needed for study. A sample size of 750 patients was estimated to give a minimum power of 90 % (type II error < 0.10) to detect a difference of total WMS score of 3 points (5 %) between the two groups. We used specially designed forms to collect data. One form was used to record descriptive information copied from medical and preanaesthetic evaluation records, such as a history of heart disease, hypertension or lung disease. We also noted details of the anaesthetic technique, the patient's recovery and postoperative complications. All personnel were instructed to register events with hypoxaemia and changes in patient care as a consequence of the oximeter monitoring. Hypoxaemia was defined as Sp_{O₂} ≤ 90 % or cyanosis, Pa_{O₂} ≤ 7.8 kPa or Sa_{O₂} ≤ 90 %. WMS results were recorded on a special WMS form; RT test results were stored on com-

TABLE III. Median scores (ranges) on the Wechsler Memory Scale (WMS) for 358 patients who had (group I), and 378 patients who did not have (group II), perioperative monitoring with pulse oximetry. *Difference calculated for each patient. None of the differences between WMS I and WMS II or between the two groups was significant

Subtest	Maximum score	Preoperative score (WMS I)		Postoperative score (WMS II)		Difference (WMS I - WMS II)*	
		Group I	Group II	Group I	Group II	Group I	Group II
Personal and current information	6	6 (4-6)	6 (5-6)	6 (5-6)	6 (5-6)	0 (-1 to 2)	0 (-1 to 1)
Orientation	5	5 (4-5)	5 (4-5)	5 (4-5)	5 (4-5)	0 (-1 to 1)	0 (-1 to 1)
Mental control	9	7 (2-9)	7 (3-9)	7 (2-9)	8 (3-9)	0 (-4 to 6)	0 (-4 to 4)
Logical memory	23	10 (2.5-18)	10 (2.5-18)	10.5 (3.5-19.5)	11 (4.5-18)	1 (-6 to 8)	1 (-5 to 8)
Memory span	15	10 (6-15)	10 (6-15)	10 (5-14)	10 (4-15)	0 (-5 to 5)	0 (-5 to 4)
Visual reproduction	14	12 (3-14)	12 (2-14)	11 (1-14)	12 (3-14)	0 (-8 to 8)	0 (-9 to 7)
Associate learning	21	13 (4-20)	13 (4-21)	11.5 (2-20)	12 (1.5-20)	-1 (-12 to 8)	-1 (-11 to 8)
Total WMS score	93	61.0 (39-81.5)	62.5 (37-83)	60.5 (34-82.5)	62.0 (37-81.5)	-1.0 (-21.5 to 16.5)	-0.5 (-17.5 to 14.5)

TABLE IV. Scores for the Continuous Reaction Time (RT) test for 358 patients who had (group I), and 378 patients who did not have (group II), perioperative monitoring with pulse oximetry. *Difference calculated for each patient. None of the differences between RT I and RT II or between the two groups was significant

	Preoperative score (ms) (RT I)		Postoperative score (ms) (RT II)		Difference (ms) (RT II - RT I)*	
	Group I	Group II	Group I	Group II	Group I	Group II
10 % fractile	232	228	239	234	6	6
Median	270	273	283	277	8	9
90 % fractile	363	356	376	365	12	11
Mean	296	292	307	301	12	8

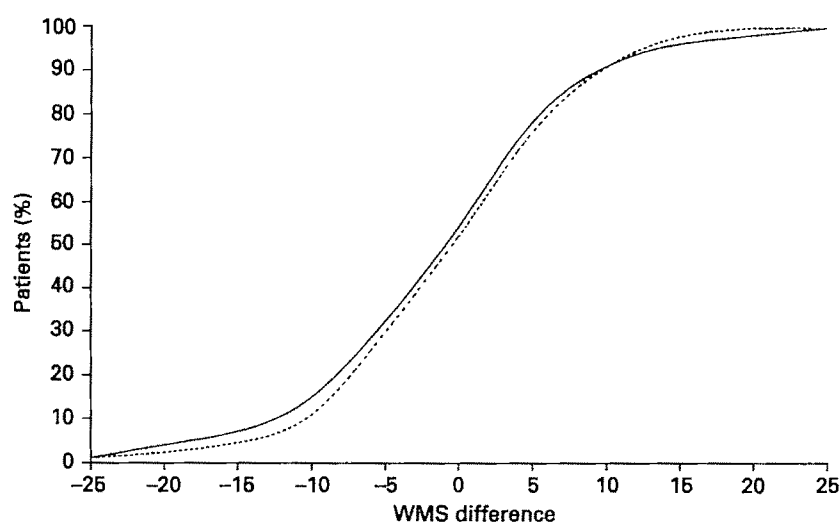


FIG. 1. Distribution of differences between preoperative and postoperative total scores on the Wechsler Memory Scale (WMS) for 358 patients who were monitored with pulse oximetry (solid line) and for 378 patients who were not (dashed line).

puter. All data were entered into a mainframe computer (IBM 4361) with specially designed management software that constructed an event history data base. The computer software used for statistical analysis included SCIBAS [24] and BMDP (BMDP Statistical Software, Inc., Los Angeles, CA). Patient data, events with hypoxaemia, changes in patient care and results from the questionnaire underwent chi-square testing or Fisher's exact test. WMS and RT data were subjected first to the Mann-Whitney test and second, to stratification by age, weight, ASA physical status and type of anaesthesia. $P < 0.05$ was considered statistically significant.

RESULTS

Initially, we enrolled 861 patients in the study for preoperative cognitive testing (WMS I and RT I). Of these, 736 (86%) completed the postoperative testing (WMS II and RT II). The other 125 patients (14%) did not complete the study: five needed to undergo a second operation, 63 refused to participate after the initial testing and 57 were discharged from hospital before postoperative testing could be administered. These 125 eliminated patients were distributed equally between the two groups and did not differ significantly from the 736 study participants in patient characteristics—risk factors (including ASA physical status), type of anaesthesia or surgical procedure and frequency of postoperative complications (10% for the total population *vs* 11% for the

eliminated group (ns)). In the subsample (the case-control part of the study), 23 patients did not return for the 3-month follow-up test, 14 being in the case group and nine in the control group.

The study population comprised 410 women and 326 men, distributed equally between the two study groups. Although patients in group I (with oximetry) ($n = 358$) were significantly heavier than group II (without oximetry) ($n = 378$) (table I), there were no other differences in risk factors or type of anaesthesia or surgical procedure (table II).

During anaesthesia, hypoxaemia was diagnosed in 7.8% of the patients in group I (with oximetry) and in 0.3% in group II (without oximetry) ($P < 0.00005$). In the recovery room, 11.7% of patients in group I and 0.5% of those in group II were diagnosed as having experienced hypoxaemia ($P < 0.00005$). The staff stated that in 24.9% of the patients the readings from the oximeter prompted one or more changes in treatment. The flow rate of supplementary oxygen given in the recovery room was significantly greater in group I, with 45% of the oximeter monitored patients receiving more than 3 litre min^{-1} of oxygen, compared with 35% of patients in group II ($P < 0.01$). The proportion of patients discharged from the recovery room with a request for supplementary oxygen was 13% in group I and 3% in group II ($P < 0.00005$).

Preoperative scores on the WMS I and RT I did not differ between groups (tables III, IV). Administration of the postoperative cognitive test occurred

TABLE V. Subjective reports (by questionnaire) of cognitive deficits. Group I: Monitored with pulse oximeter; group II: no pulse oximeter monitoring. In addition to the listed questions, the patients were asked about former problems with memory, concentration and irritability

Main questions	Group I (n = 345)		Group II (n = 374)		P
	No.	%	No.	%	
After discharge from hospital and returning to your home have you experienced problems with recent memory? (i.e. what to buy when shopping, telephone numbers, appointments)	24	7.0	41	11.0	0.07
After discharge from hospital and returning to your home have you experienced problems with ability to concentrate? (i.e. when reading your newspaper, attention to the news in the television)	36	10.4	35	9.4	0.60
After discharge from hospital and returning to your home have you experienced problems with irritability? (chiding about nothing, fly into temper for nothing)	27	7.8	42	11.2	0.12

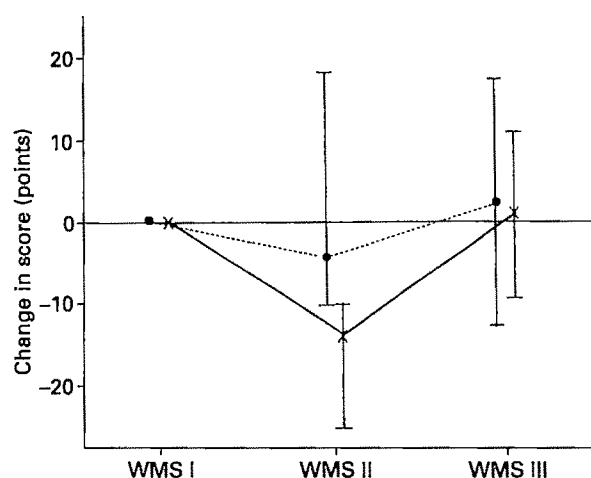


FIG. 2. Median change (ranges) in Wechsler Memory Scale (WMS) test scores for patients with suspected postoperative cognitive dysfunction ("cases") (x, solid line) and for control patients (●, dashed line). "Cases" designates patients whose WMS scores 5 days (median values) after surgery were 10 points less than preoperative scores. Control patients were selected randomly from the remaining patients and their data represent the normal postoperative variation in cognitive scores. Change in WMS score was measured at three times: before surgery (base line, WMS I); 5 days (median value) after surgery (WMS II); and 96 days (median value) after surgery (WMS III).

5 days after anaesthesia (median value; mean 5.5 days, range 2–16 days); this value did not differ between groups. For both the total score and the scores for each subtest, the median difference between preoperative (WMS I) and postoperative (WMS II) memory test results did not differ significantly between group I and group II (table III); however, the distribution of the individual differences in total scores on the two WMS tests varied widely in both study groups (fig. 1). For 17% of the patients, total WMS II scores were either 10 points greater or 10 points less than the WMS I score—a percentage that did not differ significantly between the two groups (fig. 1). The continuous reaction times tested after operation were of greater duration by a few milliseconds than before operation, but these differences were not statistically significant, nor were there significant differences between group I and group II (table IV). Strati-

fication of data by age, weight, ASA physical status and type of anaesthesia did not reveal any statistically significant difference in cognitive function between groups.

The questionnaire was returned by 97.7% of the study population 45 days after anaesthesia (median value; mean 50 days, range 40–106 days) (table V). For all patients, including the patients in the case-control part of the study, subjective perception of cognitive dysfunction did not correlate with WMS or RT test scores.

In the subsample (the case-control part of the study), 40 cases and 45 controls were tested 96 days after anaesthesia (median value; mean 97 days, range 65–140 days). There were no significant differences in the case group's characteristics concerning monitoring with or without pulse oximetry, age and type of anaesthesia, compared to the main study group (736 patients). The case group's total median WMS score on the 3-month follow-up test returned to the preoperative value. The interindividual difference for the case group was within the range of the interindividual difference for the control group (fig. 2).

DISCUSSION

In the past 30 years, several investigators have examined cognitive function after anaesthesia and surgery [1–3, 25–30]. More recent studies have concentrated on differences between the effects of general anaesthesia and those of regional anaesthesia [8, 31–37] and neuropsychological alteration after cardiac operation [28, 38, 39]. Some investigators have concluded that anaesthetic technique has no major influence on cognitive function beyond the first 3–4 days after operation [29–31]. Our results suggest that use of pulse oximetry monitoring during anaesthesia and in the recovery room does not affect cognitive outcome.

Catastrophic hypoxaemia is a well known cause of brain dysfunction [6, 7, 40]. However, several neurological studies making use of the WMS tests have demonstrated that mild to moderate hypoxaemia is also associated with cognitive dysfunctions [11–14, 41]. Did the patients in the present study experience hypoxaemia? Paradoxically, it seems as if

the patients in the pulse oximetry group suffered more hypoxaemia than the control group, in both the operating theatre and the recovery room. However, in reality hypoxaemia was discovered much more frequently in the pulse oximetry group and was treated, while in the control group it was infrequently detected and treated. From our previous blinded observational studies, we know that 75 % and 95 % of hypoxaemic episodes in the operating theatre and recovery room remain undiscovered [15, 17]. In the operating theatre 53 % and in the recovery room 55 % of these patients had one or more episodes of mild hypoxaemia with oxyhaemoglobin saturation (Sp_{O_2}) between 86 % and 90 %. Severe hypoxaemia with Sp_{O_2} less than 81 % was observed in 20 % of the patients in the operating theatre and 13 % in recovery [15, 17]. Findings of other studies during anaesthesia and postoperative recovery support our results [16, 18, 20]. In a recent, randomized study we have demonstrated that monitoring with pulse oximetry could substantially reduce the incidence, duration and severity of hypoxaemia during and after anaesthesia [19]. In this study, the incidence of hypoxaemia (Sp_{O_2} 81–85 %) in patients monitored with oximetry was three to five times less than in the control group. In the control group 7 % of the patients in the recovery room experienced $Sp_{O_2} < 80$ %, while none had such decreased values in the oximeter group [19]. Similar observations were reported in other studies [18, 20]. In the present study and in our previous reports, oximetry monitoring resulted in several changes of patient care [21, 22]. In group I (with oximetry) flow rates of supplementary oxygen in the recovery room were increased significantly and significantly more patients were discharged from recovery receiving supplementary oxygen compared with group II (without oximetry). Thus ample evidence points to a substantial difference in the incidence and severity of hypoxaemia in patients monitored with pulse oximetry compared with patients not monitored in the operating theatre and recovery room. In our study, this was not reflected in differences in the results of objective psychological tests completed by our patients. Perhaps the postulated effect of oximetry and treatment with oxygen was confounded by the well known fact that hypoxaemia is a common occurrence several days after operation—that is, at a time when none of our patients was monitored with pulse oximetry [42, 43].

In the present study, the difference between the two study groups at 6 weeks after anaesthesia regarding *subjective* perception of a cognitive dysfunction (problems with recent memory) approached, but did not reach, statistical significance ($P = 0.07$) (table V). If this difference did not arise by chance, it could represent a type II error because of inclusion of too few patients in the study. On the basis of the responses to the questionnaire, our probability of having a type II error is 53 %. It is impossible to determine the probability of a type II error (β) for the *objective* test (the WMS and the RT test) as there was no meaningful numerical difference between the two groups. However, with 736 patients in the study, the probability of a type II error was

less than 10 % if an increase of 3 points (5 %) in the total WMS score of the pulse oximetry group had been achieved. This agrees well with the required minimum statistical power of 90 % established in the design of the trial. To test the hypothesis that non-catastrophic perioperative hypoxaemia affects the patient's *subjective* perception of mental function, a larger group of patients than selected by us would be required, or we would need to apply *objective* tests to patients at special risk of hypoxaemia-induced cognitive dysfunction. Patients with systemic diseases (ASA physical status III or IV) or elderly patients already suffering cognitive dysfunction may be more sensitive to the putative effects of non-catastrophic hypoxaemia. The fact that stratification of data by age and ASA physical status did not reveal any difference between groups does not argue against this hypothesis, as only 7 % of our study patients were classified ASA III or IV (table II) and only 43 % of the patients were older than 60 yr.

The scores of individual patients varied widely between preoperative and postoperative tests, this tendency being most evident with the WMS (fig. 1). The decrease in the total postoperative WMS scores of more than 50 % of the patients was nearly equalled by the increase in scores of the others. Normal biological variation and many confounding factors may influence the results of postoperative cognitive tests. An increase in scores could have resulted from previous exposure to a given test and from the recovery from illness (the patient would be free of pain or less anxious). A decrease in scores could result from a residual or direct effect of anaesthesia; from sedation, intraoperative hyperventilation or analgesics; or from surgery itself and the hormonal or metabolic sequelae [3, 26, 27, 30, 37, 44]. To reduce the influence of these confounding factors, our postoperative cognitive tests were administered at the day of discharge from hospital or at the latest on the 7th day after operation. The WMS was chosen to reduce the effects of practice, as this test exists in well standardized parallel forms. Furthermore, before the first RT test, a learning session familiarized the patient with the procedure. Finally, we excluded patients with suspected or confirmed cancer as a consequence of our own pilot study and other previous reports [45, 46] suggesting that cancer itself is associated with cognitive disorders in addition to well known affective changes such as depression.

Group analysis of the results may conceal important individual variation in the case-control part of the study [47]. We followed the patients who had the greatest decrease in postoperative test scores and included a control group to document normal postoperative variation in cognitive scores (fig. 2). Three months after anaesthesia and surgery, the case group's median WMS score had returned to the preoperative value. This observation is supported by results from other recent studies [37–39, 44]. However, a practice effect may have influenced the follow-up test. The fact that the WMS test exists in only two parallel forms obliged us to reuse the preoperative WMS form at the follow-up. We believe this bias did not influence the retest which

occurred after 3 months. This is supported by the fact that the control group experienced only a slight and insignificant improvement in test score at the follow-up test (WMS III) (fig. 2).

One may speculate that application of a broader range of neuropsychological assessments than used in the present study could have detected varying deficits of an enduring nature. Using a broad range of tests, several investigators have described moderate to severe cognitive dysfunction lasting for several months after coronary bypass surgery [28, 38, 39]. Interestingly, several of these cognitive dysfunctions were detected with the WMS test [28, 38]. Although 9% of our total population thought that mental functioning had deteriorated, this perceived deficit was not confirmed by objective tests. Another study reported similar results (a perceived but unconfirmed cognitive deficit) 3 months after anaesthesia [44]. This finding suggests that some of the widely used psychological tests may not be sensitive enough to detect changes in cognitive function reported by a patient.

The drop-out rate of subjects in our study (14%) is a cause of concern, especially if the loss were caused by postoperative complications that could influence cognitive function. However, analysis of the patient data, risk factors, types of anaesthesia and surgical procedures, and preoperative cognitive test results revealed no significant difference between the study population and the patients who were eliminated from study. Most importantly, the incidence of postoperative complications was the same for the two groups, suggesting that loss of subjects did not bias our results.

We believe the present study raises several questions that future studies should examine. What caused 9% of our patients to perceive that their memories were impaired 6 weeks after anaesthesia and operation? Is it possible that hypoxemia, which occurs commonly during the first days after operation [42, 43], played a role in the cognitive impairment reported by our patients? Future studies must assess the possible contribution of postoperative hypoxaemia to cognitive dysfunction. May some other objective tests of cognition confirm the subjective impressions reported by our patients? Should future studies concentrate on elderly patients sensitive to cognitive impairment and undergoing major operations under general anaesthesia?

In summary, for our 736 patients, subjective and objective measures did *not* indicate less late postoperative cognitive impairment after perioperative monitoring with pulse oximetry, and the 3-month follow-up test did not produce any objective signs of major cognitive dysfunction in any patient.

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UTEROPLACENTAL AND FETAL CIRCULATION DURING EXTRADURAL BUPIVACAINE–ADRENALINE AND BUPIVACAINE FOR CAESAREAN SECTION IN HYPERTENSIVE PREGNANCIES WITH CHRONIC FETAL ASPHYXIA

S. ALAHUHTA, J. RÄSÄNEN, P. JOUPPILA, R. JOUPPILA AND A. I. HOLLMÉN

SUMMARY

We have studied the effects of an extradural block during Caesarean section using either bupivacaine plain or with adrenaline 85–100 µg on blood velocity waveforms of maternal uterine and placental arcuate arteries and fetal umbilical, renal and middle cerebral arteries, in 20 hypertensive parturients with chronic fetal asphyxia. Fetal myocardial function was investigated at the same time by M-mode echocardiography. Extradural anaesthesia resulted in a significant decrease in maternal mean systolic and diastolic arterial pressures in both groups, but this was more marked after plain bupivacaine. There were no significant differences in any of the Doppler recordings relative to baseline values after plain bupivacaine, but after bupivacaine with adrenaline there were significantly increased blood flow velocity indices for the maternal uterine and placental arcuate arteries and significantly decreased indices in the fetal renal and middle cerebral arteries. Neonatal outcome as evaluated by Apgar scores and acid–base values in the umbilical cord were similar in the two groups. The results suggest that adrenaline added to the solution of bupivacaine increased vascular resistance in the uteroplacental circulation, indicating impaired blood flow. (Br. J. Anaesth. 1993; 71: 348–353)

KEY WORDS

Anaesthesia, obstetric: Caesarean section. Anaesthetic techniques: extradural. Placenta: umbilical blood flow. Uterus: blood flow.

Most obstetric anaesthetists regard extradural anaesthesia as the method of choice for Caesarean section in hypertensive disorders of pregnancy [1]. Unlike general anaesthesia, it provides a stable maternal haemodynamic state in severely toxæmic parturients [2] and pain relief may be accomplished easily after operation, either with extradural local anaesthetics or, preferably, by combining these with opioids [3].

The Duplex ultrasonic method, combining real-time and Doppler techniques and using a colour flow system, allows detailed examination of maternal and fetal haemodynamics. We have used this method recently and found that extradural anaesthesia with

either bupivacaine or bupivacaine combined with adrenaline for Caesarean section did not cause marked changes in the uteroplacental or fetal circulation of normotensive mothers with a non-asphyxiated fetus [4, 5]. In the present study we have performed a similar investigation in hypertensive parturients with chronic fetal asphyxia.

PATIENTS AND METHODS

After obtaining approval from the local Ethics Committee and informed consent, we studied 23 parturients in gestational weeks 28–36 undergoing Caesarean section under extradural anaesthesia. None of the patients was in labour. Inclusion criteria were gestational hypertension and chronic fetal asphyxia as indicated by increased blood flow velocity waveform indices for the umbilical artery, greater than 2 SD of our normal material in repeated examinations. Exclusion criteria were multiple gestation, other maternal medical complications except for hypertension or pre-eclampsia and standard contraindications to extradural anaesthesia.

Each subject received 0.3-mol litre⁻¹ sodium citrate 30 ml orally. An extradural catheter was inserted at the L2–3 or L3–4 interspace with the patient in the left lateral position, using an 18-gauge needle and loss of resistance to air. The patient was placed in the supine position with a wedge under the right hip after the catheter had been advanced 3 cm cephalad into the extradural space. All the patients received oxygen 3 litre min⁻¹ via nasal cannulae throughout; ECG was monitored continuously and maternal arterial pressures and heart rate were recorded every 5 min using automated, non-invasive equipment. Mean maternal heart rate and arterial pressures were calculated for four periods: from entry into the operating theatre to the test dose; during establishment of the extradural anaesthesia; during the second ultrasound recording; and from its completion to the delivery.

Subjects were allocated randomly by means of

sealed envelopes, to receive either 0.5% bupivacaine with adrenaline 1:200 000 or 0.5% bupivacaine plain. Only the anaesthetist (S.A.) performing the blocks knew which solution was used. An initial test dose of 3 ml was followed by incremental doses of the same solution until a block up to T4 was obtained as assessed by pinprick. If the sensory block was below T4 after bupivacaine-adrenaline 20 ml, additional doses of 0.5% plain bupivacaine were given. Preloading was accomplished by infusing 1 litre of balanced electrolyte solution i.v. During the second Doppler recording and subsequently, fluid administration was titrated according to the maternal haemodynamic responses and to maintain urine output. A reduction in systolic arterial pressure of more than 20% relative to control or a systolic arterial pressure less than 110 mm Hg was treated by increasing the infusion. If this was ineffective, ephedrine 5 mg i.v. was administered.

Ultrasound measurement of uteroplacental and fetal haemodynamic variables were performed by the same obstetrician (J.R.) on two occasions: after insertion of the extradural catheter and before injection of the local anaesthetic (control values), and when sensory analgesia had reached the T4 level. Each patient provided her own baseline readings. All the recordings were made with the subject in the left semi-lateral position during periods of fetal rest and apnoea. The equipment and methodology used have been described previously [4]. Briefly, a colour flow mapping system with a 3.75-MHz, pulsed-wave Doppler sector transducer (Toshiba, model SSA-270A) was used to obtain blood flow velocity waveforms for the maternal uterine artery (main branch on the placental side of the uterus), the placental arcuate artery and the fetal umbilical, middle cerebral and renal arteries, using the average of three waveforms. Arterial resistance was expressed in terms of the pulsatility index (PI), calculated automatically and defined as the difference between the peak systolic and end-diastolic values divided by the averaged maximum flow velocity of the cycle. M-mode echocardiographic examination of the fetal heart was performed using the same ultrasonic scanner and probe. The four-chamber view of the fetal heart was imaged at the level of the inflow tracts. The end-systolic and end-diastolic inner diameters of the ventricles were recorded from the M-mode image and used to calculate the fractional and mean circumferential shortening of the myocardium [6]. The inner end-diastolic diameters were used to determine the size of the fetal heart and the ventricular fractional and mean circumferential shortenings, to evaluate its functional properties. The aortic and pulmonary valves were visualized from the five-chamber and short axis views. Valve flow was ascertained by means of colour Doppler. The pulsed Doppler sample volume was placed just above the valve leaflets, and velocity waveforms were measured over three consecutive cardiac cycles and averaged. Recordings with an angle larger than 5° between the transducer beam and the pulmonary trunk and aorta were rejected.

Blood samples after delivery of the infant were obtained from a double-clamped umbilical cord vein

and artery for measurement of acid-base status. Apgar scores were allocated by a paediatrician who was unaware of the local anaesthetic used.

Statistical comparisons between the groups were performed using two-sample *t* tests and those within the groups by the two-tailed *t* test for paired data. $P < 0.05$ was accepted as statistically significant. Values are expressed as mean (SD).

RESULTS

Three patients were excluded from analysis because of normal umbilical artery PI values in the control measurement. In the remaining 20 subjects, PI for

TABLE 1. Maternal characteristics (mean (range or SD))

	Bupivacaine (n = 10)	Bupivacaine- adrenaline (n = 10)
Age (yr)	32.4 (21–44)	32.6 (22–40)
Weight (kg)	66 (5.7)	65.3 (5.5)
Height (cm)	161.7 (4.4)	162.5 (4.7)
Gestational weeks (wk)	33.7 (2.7)	32 (3.1)
Parity		
Primiparae	5	5
Multiparae	5	5
Labetalol therapy	6	7

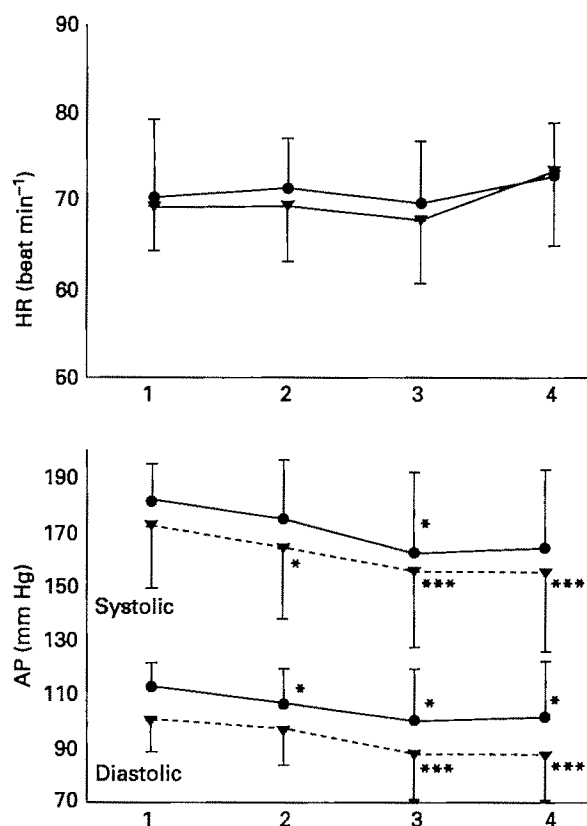


FIG. 1. Mean (SD) maternal heart rate (HR) and arterial pressure (AP) before extradural anaesthesia (1), during its establishment (2), during the second ultrasound measurement (3), and after it up to delivery (4). ● = Bupivacaine with adrenaline; ▼ = plain bupivacaine. Significant differences from control: * $P < 0.05$; *** $P < 0.001$.

TABLE II. Mean (SD) fetal heart rate and blood velocity waveform indices (pulsatility index) for the maternal uterine, placental arcuate and fetal umbilical, middle cerebral and renal arteries before and after extradural anaesthesia with bupivacaine-adrenaline and plain bupivacaine

	Bupivacaine-adrenaline			Bupivacaine		
	Before extradural	After extradural	P	Before extradural	After extradural	P
Fetal heart rate (beat min ⁻¹)	136 (6.3)	129 (7.8)	0.072	138 (10.8)	134 (9.3)	0.188
Pulsatility index						
Uterine artery	1.20 (0.36)	1.52 (0.55)	0.003	1.34 (0.35)	1.48 (0.45)	0.121
Arcuate artery	0.56 (0.11)	0.66 (0.11)	0.005	0.76 (0.25)	0.78 (0.21)	0.791
Umbilical artery	2.14 (1.38)	2.02 (1.67)	0.324	2.15 (1.50)	1.81 (0.66)	0.346
Middle cerebral artery	1.60 (0.47)	1.38 (0.39)	0.029	1.40 (0.44)	1.41 (0.43)	0.881
Renal artery	2.67 (0.46)	2.11 (0.54)	0.037	2.26 (0.50)	2.25 (0.39)	0.936

TABLE III. Changes in fetal myocardial contractility and ventricular sizes (mean (SD)) before and after extradural anaesthesia with bupivacaine-adrenaline and plain bupivacaine. IDD = inner end-diastolic dimensions

	Bupivacaine-adrenaline			Bupivacaine		
	Before extradural	After extradural	P	Before extradural	After extradural	P
Left ventricle						
Fractional shortening (%)	34.2 (4.3)	33.4 (3.7)	0.373	31.3 (4.9)	31.9 (2.9)	0.526
Mean circumferential shortening (circ s ⁻¹)	1.34 (0.21)	1.25 (0.20)	0.203	1.31 (0.20)	1.27 (0.21)	0.349
IDD (mm)	12.2 (2.7)	12.6 (2.8)	0.241	13.8 (1.8)	13.9 (1.8)	0.808
Right ventricle						
Fractional shortening (%)	28.0 (5.5)	27.3 (3.5)	0.717	28.6 (4.7)	28.0 (5.4)	0.616
Mean circumferential shortening (circ s ⁻¹)	1.06 (0.19)	1.00 (0.13)	0.358	1.20 (0.21)	1.12 (0.28)	0.292
IDD (mm)	14.4 (3.4)	13.8 (3.0)	0.097	15.5 (1.9)	15.2 (2.2)	0.589
Pulmonary trunk						
Systolic peak velocity (cm s ⁻¹)	68.6 (12.5)	63.9 (7.3)	0.201	67.3 (12.4)	67.4 (12.2)	0.948
Ascending aorta						
Systolic peak velocity (cm s ⁻¹)	76.1 (13.4)	79.0 (8.7)	0.460	82.6 (15.2)	82.2 (15.2)	0.893

the umbilical artery was ≥ 2 SD greater than the normal range or had absent or reverse end-diastolic flow in repeated measurements. These 20 patients were divided equally between the groups. The bupivacaine-adrenaline group comprised seven patients with pre-eclampsia, one with chronic hypertension and two with gestational hypertension (one of whom was proteinuric). Of the patients receiving plain bupivacaine, six were diagnosed as having pre-eclampsia and four as having gestational hypertension without proteinuria [7]. Hypertension was defined as two consecutive measurements, 4 h apart of a diastolic arterial pressure exceeding 90 mm Hg. The five pregnancies with pre-eclampsia in each group were severe, as demonstrated by a diastolic arterial pressure of 110 mm Hg or more on at least two occasions 6 h apart and proteinuria of at least 5 g/24 h. There were no significant differences in mean age, weight and height of the parturients or gestational weeks between the two groups (table I).

The dose of adrenaline administered with extradural bupivacaine varied between 85 μ g and 100 μ g, except for one patient to whom 150 μ g was given in

error. Mean doses of bupivacaine were 23.8 (3.4) ml and 24.6 (7.3) ml in the plain and bupivacaine-adrenaline groups, respectively.

Mean maternal heart rate remained stable throughout in both groups, but extradural anaesthesia resulted in significant decreases in mean maternal systolic and diastolic arterial pressures in the bupivacaine-adrenaline group. A similar change was observed in the patients receiving plain bupivacaine, but the decrease was more marked, being significant even during institution of the block and after the second Doppler examination while, after bupivacaine-adrenaline, mean maternal systolic and diastolic arterial pressures returned towards baseline values at the beginning of surgery (fig. 1). The decrease in maternal arterial pressures was easily controlled and no patient exhibited profound hypotension. No vasopressors were needed and all the parturients had an uneventful postpartum recovery.

There were no significant differences in any of the PI values relative to baseline after plain bupivacaine, whereas the mean maternal uterine and arcuate PI

TABLE IV. Neonatal characteristics (mean (SD))

	Bupivacaine	Bupivacaine-adrenaline
Neonatal weight (g)	1698 (508)	1394 (599)
Umbilical arterial pH	7.27 (0.03)	7.28 (0.03)
Umbilical venous pH	7.31 (0.03)	7.34 (0.03)
Apgar scores		
1 min < 7	0	1
5 min < 7	1	0

values increased significantly in those who received bupivacaine-adrenaline. The mean PI of the fetal renal and middle cerebral arteries decreased significantly after bupivacaine-adrenaline (table II). There were no significant differences relative to control in either the fetal myocardial measurements or the systolic peak velocities of the pulmonary trunk and ascending aorta in both groups (table III). The fetal heart rate was not affected by administration of local anaesthetic.

Five parturients with breech presentation were given general anaesthesia with inhalation anaesthetics in addition to extradural anaesthesia at the obstetrician's request, to facilitate delivery by inducing uterine relaxation. The perinatal data in these babies were excluded.

The mean birthweights of the neonates were 1698 (508) g and 1394 (599) g in the plain bupivacaine and bupivacaine-adrenaline groups, respectively. The mean pH values in the umbilical arterial and venous blood were 7.27 (range 7.22–7.31) and 7.31 (range 7.26–7.36) after plain bupivacaine, and 7.28 (range 7.24–7.31) and 7.34 (range 7.30–7.39) after bupivacaine-adrenaline. One neonate in the bupivacaine-adrenaline group at 1 min, and one in the plain bupivacaine group at 5 min had Apgar scores less than 7. There were no significant differences in neonatal characteristics between the groups (table IV). All the infants were transferred to the neonatal intensive care unit after birth. Two of those in the bupivacaine-adrenaline group (birthweights 830 g and 660 g) died because of respiratory distress syndrome on the 4th day postpartum; these deaths were not attributable to anaesthesia.

DISCUSSION

We have found that, when hypertensive pregnancy is complicated by chronic fetal asphyxia reflected as high umbilical vascular resistance, adrenaline combined with bupivacaine further increased Doppler velocity indices in the maternal uterine and placental arcuate arteries. This indicates increased resistance and impaired uteroplacental blood flow [8]. Our results are in agreement with those of Rosenfeld, Barton and Meschia, who demonstrated a reduction in total uterine blood flow in spite of an unaltered mean arterial pressure in the pregnant ewe during a slow, constant infusion of adrenaline [9]. In the present study, maternal mean arterial pressures decreased significantly after extradural bupivacaine-adrenaline, but no major hypotensive

episodes occurred. Thus no real decrease in uteroplacental perfusion as a result of a reduction in maternal arterial pressure seems likely.

Although the parturients represented various manifestations of gestational hypertensive disorders, they were comparable in that all the fetuses were suffering from chronic asphyxia as demonstrated by increased blood flow velocity waveform indices for the umbilical artery. In spite of fetal distress and a reduction in uteroplacental blood flow induced by adrenaline, there were no significant changes in Doppler measurements from the fetal umbilical artery. Animal experiments suggest that the healthy fetus has a wide safety margin with respect to uteroplacental haemodynamic disturbances [10]. This safety margin may not exist, however, for the growth-retarded, chronically asphyxiated human fetus, and even a minimal reduction in uteroplacental perfusion may be deleterious to a compromised fetus, although such a relationship was not demonstrated in the present trial. The fetal cardiovascular response to hypoxia is blood flow redistribution to the central organs, which is reflected in decreased vascular resistance in the fetal middle cerebral artery [11]. Our observed decrease after bupivacaine-adrenaline was unlikely to have been a result of hypoxia, as the vascular resistance also diminished in the fetal renal artery, which reflects peripheral resistance. Although adrenaline with either lignocaine [12] or bupivacaine [5] does not have any influence on umbilical vascular resistance or the fetal circulation in healthy term gravidae, a reduced activity of the catecholamine-metabolizing enzymes, monoamine oxidase [13] and catechol-O-methyl transferase [14], observed in the placenta of patients with chronic hypertension and toxemia could lead to an increased passage of adrenaline from mother to fetus and account for these changes in the fetal vessels.

Local anaesthetics containing adrenaline have been used previously in hypertensive disorders of pregnancy. Heller and Goodman were the first to describe patients with pre-eclampsia in whom a local anaesthetic with adrenaline was used for both labour analgesia and Caesarean section [15, 16]. None of the cases showed a maternal hypertensive reaction or fetal distress or adverse neonatal effects as judged by continuous fetal heart rate monitoring and Apgar scores. In addition, Dror and colleagues administered 40 µg of adrenaline with lignocaine for labour analgesia to mildly pre-eclamptic patients and noted no maternal hypertension [17]; on the contrary, arterial pressures declined after lignocaine-adrenaline. The authors of these articles postulated that adrenaline absorbed slowly from the extradural space induces primarily beta₂-adrenergic vasodilatation and a decrease in mean systolic and diastolic arterial pressures—the same effect as demonstrated in non-pregnant volunteers [18]. In contrast, we have found that hypotension was less marked with bupivacaine-adrenaline than with the plain solution. Adrenaline increases systolic arterial pressure more than diastolic [19], as also observed here as a less marked decrease in mean systolic arterial pressures.

The relationship between maternal, uteroplacental and fetal haemodynamic responses and adrenaline is confounded by our finding that seven of 10 patients in the bupivacaine-adrenaline group were receiving oral labetalol, which selectively blocks α_1 -adrenoceptors and non-selectively blocks β_1 - and β_2 -adrenoceptors [20]. The ratio between its α - and β -blocking effects is estimated to be 1:3 with oral administration. Labetalol does not seem to alter uteroplacental and fetal haemodynamics after i.v. administration in pre-eclamptic mothers [21], and it produces only minor degrees of fetal adrenergic block in the pregnant ewe [22], although it crosses the placenta easily. Labetalol therapy does not seem to prevent the effects of adrenaline on the uteroplacental and fetal circulation observed here.

Our finding of controllable hypotension after extradural bupivacaine plain in hypertensive pregnancies is comparable to those of previous investigations [2, 23]. Pre-eclampsia is a leading cause of maternal death, frequently from cerebrovascular accident [24], and control of hypertension may save lives. We found no significant changes in the Doppler recordings of the uteroplacental circulation after extradural plain bupivacaine. Previous research has demonstrated an increase in intervillous blood flow [25] and a decrease in velocity waveform indices of uterine and umbilical arteries during hypertensive disorders of pregnancy [26, 27]. However, the patients in both these studies were in active labour, while ours were not. Plasma concentrations of catecholamines are greater in pre-eclamptic than in normal parturients during labour and these are reduced significantly after extradural analgesia [28]. The reduction in sympathoadrenal activity during labour could explain the decrease in uteroplacental vascular resistance, which did not occur in our non-labour patients. The maintenance of uteroplacental perfusion in our study was beneficial, as even these chronically distressed fetuses were not jeopardized by the anaesthetic technique, as confirmed also by blood velocity indices in the fetal circulation, which remained unchanged. Furthermore, there were no depressant effects of bupivacaine on fetal myocardial variables, even though relatively large doses of local anaesthetic were used.

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RESPIRATORY SINUS ARRHYTHMIA: A NEW, OBJECTIVE SEDATION SCORE

D. Y. WANG, C. J. D. POMFRETT AND T. E. J. HEALY

SUMMARY

We tested if microcomputer-based measurements of heart rate variability and respiratory sinus arrhythmia (RSA) could be used as the basis of an objective sedation score. Measurements were obtained in eight ICU patients before, during and after physiotherapy. Patients were sedated with propofol and alfentanil and paralysed with atracurium. Mean ECG R-R interval showed little variation, changing from 646.15 (SD 203.15) ms to 596.08 (181.75) ms and 633.98 (184.53) ms before, during and after physiotherapy, respectively (not significant). However, the degree of respiratory sinus arrhythmia, determined using circular statistical analysis, increased significantly, from 0.14 (0.11) to 0.24 (0.15), during physiotherapy and returned to control after physiotherapy ($P < 0.05$). Changes in respiratory sinus arrhythmia may provide an objective measurement of sedation in ICU patients and could form the basis of a simple sedation scoring system. (Br. J. Anaesth. 1993; 71: 354-358)

KEY WORDS

Heart: respiratory sinus arrhythmia. Measurement techniques: heart rate variability, sedation.

Cardiac beat-to-beat variability (R-R variability) is a non-pathological feature of most normal ECG records, and is especially pronounced in children and young adults. The most consistent feature of heart rate variability is respiratory sinus arrhythmia (RSA), in which the heart rate increases during inspiration and decreases during expiration, through a predominately parasympathetic reflex connecting stretch receptors in the lungs and aorta to vagal motor neurones innervating the heart. Wheeler and Watkins [1] reported that there was a reduction in heart rate variability in diabetic patients exhibiting autonomic neuropathy associated with vagal denervation of the heart. Subsequent reports [2-4] confirmed that heart rate variability could be used as a clinical test in the diagnosis of diabetic peripheral neuropathy. Donchin, Feld and Porges [5] used an off-line technique to observe changes in RSA in 10 female patients during isoflurane-nitrous oxide anaesthesia, and suggested that on-line analysis of RSA might provide a physiological index of depth of anaesthesia and rate of recovery. Sakuma, Ueda and

Kiode [6] investigated heart rate variability and the function of the autonomic nervous system during general anaesthesia in 26 patients, and further concluded that heart rate variability might provide information which could be useful for monitoring depth of anaesthesia. However, the previous reports collected data over prolonged periods, such as 10 min, and have, therefore, had only low temporal resolution. This is undesirable in any putative indicator of anaesthetic depth. In addition, the earlier reports did not use real time, on-line analysis during anaesthesia, which would be required for a patient monitoring system, but depended instead on off-line analysis. Pomfrett and colleagues [7] reported changes in RSA, using on-line analysis, observed during general anaesthesia in 60 patients. Reductions in RSA during anaesthesia, together with increases in RSA during the recovery period, were identified. These results suggested that RSA, analysed on-line, may also be a useful indicator of depth of anaesthesia.

The majority of patients in the ICU are sedated and it would be useful to have an objective measurement system which could identify if sedation is either too profound or inappropriately light. The ideal system would also be simple, and able to present sedation scores to ICU staff in real-time, as a result of on-line analysis. The present study was designed to evaluate if changes in both heart rate variability and RSA could form the basis of a monitor for assessing depth of sedation in patients during intensive care, in order to develop a simple, objective sedation score.

PATIENTS AND METHODS

Informed consent was obtained from ICU patients or relatives of patients, in accordance with a research proposal approved by our local Ethics Committee.

All the patients were paralysed with atracurium and sedated with propofol and alfentanil, which were infused constantly through infusion pumps (see table I for dosage). Drug administration was maintained

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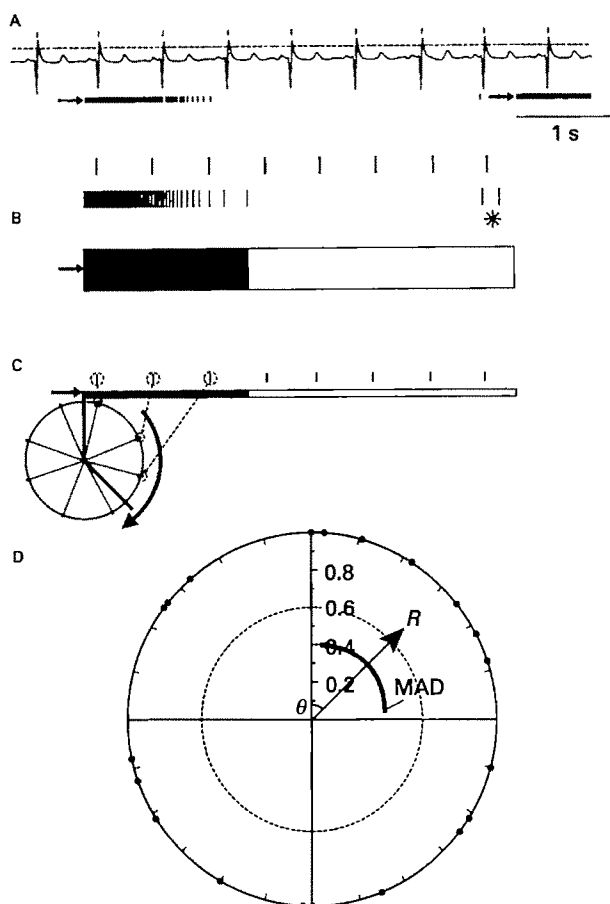


FIG. 1. Derivation of indices describing respiratory sinus arrhythmia (RSA). This schematic diagram follows the structured design of on-line, multi-tasking software developed by CJDP. A: The raw ECG was digitized at 1-KHz, R-waves were discriminated using an analog pulse trigger (threshold shown by dotted line) and the resulting R-wave pulses logged as events with timings relative to the start of each computer data file. Pulses from a flowmeter were also logged as events to 1 ms accuracy. Arrows denote the beginning of each inspiration. B: The computer determined the onset of each inspiration from the pattern of ventilatory pulses. Occasional isolated pulses (*) caused by mechanical movement of the flowmeter were rejected by the computer. The ECG R-wave event pulses were then time-normalized relative to the beginning of each ventilatory cycle. Arrow denotes the onset of inspiration, the duration of which is marked by the solid bar. C: Normalized ECG R-wave times were converted into vector co-ordinates, with a magnitude of 1 and an angle depending on their position in the ventilatory cycle (e.g. an ECG R-wave at the onset of inspiration would have an angle of 0° and a magnitude of 1, whereas an R-wave exactly half way through the ventilatory cycle would have an angle of 180° and a magnitude of 1). Filled bar denotes inspiration. D: Vectors were plotted together for successive ventilatory cycles, and a mean vector calculated. The mean vector length (R , shown $\times 10$ on the figure) was proportional to the amount of clustering of the ECG R-waves relative to inspiration, and gave an indication of RSA. The mean vector angle (θ) was also calculated. Mean angular deviation (MAD) was the circular analogue of a linear SD and indicates the distribution of vector angles about the mean vector angle.

constant for at least 2 h before the start of data collection and no additional doses were given before or during the data collection period. The patient's tracheas were intubated and their lungs ventilated during the study. Physiotherapy was used as a standardized stimulation and consisted of "bag squeezing" (manual hyperinflation of the lungs), massage of the chest, suction and passive movement of the upper and lower limbs. The procedures were

performed on all the patients by the same physiotherapist. Data were collected continuously from 15 min before physiotherapy until 15 min after physiotherapy concluded. Heart rate, core temperature and arterial $P\text{CO}_2$ were measured using standard ICU monitors.

A microcomputer-based data logging system was used to collect ECG and respiratory data, using multi-tasking software developed for the study. Details of the equipment have been published elsewhere [8]. The ECG was collected using isolated research amplifiers (Digitimer Neurolog). A turbine-based flowmeter (FDE Magtrak) was introduced into the breathing circuit and gave a pulse train during inspiration. The ECG was amplified to ± 5 V peak-to-peak amplitude and then digitized to 12-bit, 1-KHz accuracy, using a programmable laboratory interface (CED 1401). Ventilatory pulses were logged as events to 1 ms accuracy by the interface and both signals were uploaded to microcomputer (Acorn Archimedes RISC workstation) for on-line analysis and archiving of raw data.

The degree of RSA was determined using a modified version of the mean circular resultant technique described for screening diabetics exhibiting autonomic neuropathy [9] (fig. 1). The principal modification was that patients were not able to maintain a fixed duration of breathing cycle, and so successive ventilatory cycles were normalized on-line and in real-time by a computer program designed for this study. Each complete cycle of inspiration and expiration was normalized, so that it began at 0° and ended at 359° , irrespective of its duration. ECG R-wave times were logged as individual vectors within each ventilatory cycle, with magnitude 1 and angle dependent on the position in the cycle. For example, an ECG R-wave occurring at the midpoint of the ventilatory cycle would be represented by a vector with an angle of 180° and a magnitude of 1. The following variables were obtained (fig. 1):

R : the mean vector length of several vectors of the ECG R-waves recorded during a 30-s epoch. The magnitude of each mean vector describes the degree of clustering of the R-wave distribution within a ventilatory cycle, and hence the degree of RSA. The range of its value is from 0 to 1.

P : the 0.05 probability level for Rayleigh's test of randomness [10]. If R exceeds P , there is a significant increase in the amount of clustering of the ECG R-waves within a ventilatory cycle and, therefore, an increase in the degree of RSA.

θ : the mean vector angle of several vectors of ECG R-waves. θ denotes the position of the mean of the ECG R-wave cluster within a ventilatory cycle. This effectively denotes the phase of RSA during ventilation.

Mean angular deviation: this is the circular statistical analogue of the linear SD, and indicates the distribution of ECG R-wave clustering about θ .

Mean R-R interval: mean duration of R-R intervals in consecutive 6-s periods, which may be considered as the instantaneous heart rate.

SD of the ECG R-R interval: the SD of the mean R-R interval has been used widely in the past as an

TABLE I. Patient characteristics and drug therapy (mean (range or SD))

No. of patients	8
Age (yr)	46.8 (23-78)
Outcome (survived/died)	6/2
Sex (M/F)	5/3
Body weight (kg)	75.9 (15.73)
Propofol (mg kg ⁻¹ h ⁻¹)	1.55 (0.65)
Alfentanil (µg kg ⁻¹ h ⁻¹)	6.91 (1.92)
Atracurium (mg kg ⁻¹ h ⁻¹)	0.79 (0.24)

index of heart rate variability [11], and contains RSA as a component. It is presented in this study in order to provide a common reference against previous studies.

All the data in the three periods (before, during and after physiotherapy) were compared using ANOVA (analysis of variance). Data files were copied onto an IBM AT-compatible computer and analysed using standard software [12]. $P < 0.05$ was considered significant.

RESULTS

We studied eight patients; two subsequently died in the ICU and six were discharged to the wards. Patient characteristics and some clinical data are shown in table I.

The changes in heart rate variability, derived parameters and haemodynamic variables are shown in table II. R increased significantly ($P < 0.01$), from 0.14 to 0.24—an increase of 71% during the period of physiotherapy. Figure 2 shows the changes in R from a single patient during physiotherapy. Mean ECG R-R interval decreased slightly, from 646.15 ms to 596.08 ms (8.0%), during physiotherapy (not significant). The SD of the ECG R-R interval increased from 29.97 ms to 79.06 ms (163.8%) during physiotherapy ($P < 0.05$) and returned to the initial value after physiotherapy. The P value of Rayleigh's test, θ and the mean angular deviation remained unchanged during physiotherapy ($P > 0.05$).

TABLE II. Cardiovascular response and heart rate variability (mean (SD)). MAD = Mean angular deviation. * $P < 0.05$ (ANOVA)

	Before physiotherapy	During physiotherapy	After physiotherapy
R	0.14 (0.11)	0.24 (0.15)*	0.15 (0.13)
P	0.34 (0.04)	0.36 (0.03)	0.35 (0.03)
θ (°)	172.93 (32.04)	174.11 (28.92)	175.03 (26.14)
MAD (°)	75.01 (4.46)	70.47 (6.44)*	74.47 (5.04)
R-R interval (ms)	644.15 (203.15)	596.08 (181.75)	633.98 (184.53)
SD of R-R interval (ms)	29.97 (24.80)	79.06 (100.48)*	21.97 (23.43)
Heart rate (beat min ⁻¹)	93.20 (6.70)	100.30 (5.80)	94.64 (5.90)
Mean AP (mm Hg)	97.20 (6.90)	106.90 (6.10)*	96.44 (5.30)

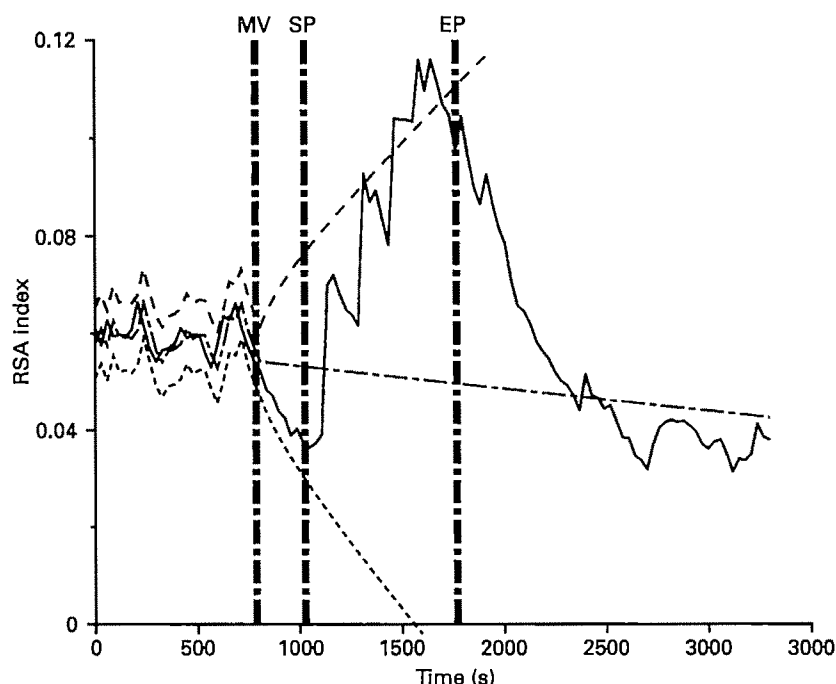


FIG. 2. ARIMA (Auto Regressive Integrated Moving Average) analysis of the circular statistical index of RSA (R (—)) against time before, during and after physiotherapy for one patient. Upper (—) and lower (---) 99% confidence intervals are shown, showing the predicted deviation from R (ARIMA R (---)) based on the behaviour of R before physiotherapy. R increased to significantly greater than ARIMA R during physiotherapy, and decreased back to the predicted, ARIMA, R value again after physiotherapy was completed. R decreased after the change to manual ventilation (MV) but this decrease did not exceed the 99% confidence interval for ARIMA R . SP = Start of physiotherapy; EP = end of physiotherapy.

Both heart rate and mean arterial pressure, taken from standard ICU monitors, increased during physiotherapy and returned to previous values after physiotherapy; changes in mean arterial pressure were significant ($P < 0.05$) but those in heart rate were not. No significant differences were observed in core temperature or arterial P_{CO_2} either between patients or during the course of individual recordings.

DISCUSSION

It has been suggested that the degree of sedation in ICU patients should be measured as routinely as ECG and arterial pressure [13], but there appears to be no objective method available. Existing scoring systems are based on subjective estimates of level of sedation, making them potentially unreliable, time-consuming and generally unsuitable for routine use in a busy ICU.

Our data suggest that changes in heart rate variability and RSA during physiotherapy are related to changes in degree of sedation in patients in the ICU. The magnitude of RSA (R) increased during the period of physiotherapy compared with baseline obtained during the period before physiotherapy. This finding is similar to that reported elsewhere during lightening of general anaesthesia. Donchin, Feld and Porges [5] reported an increase in heart rate variability during recovery from isoflurane-nitrous oxide anaesthesia. Pomfrett and colleagues [7] studied the changes in RSA in 60 patients undergoing elective surgery and showed an increase in RSA both after tracheal intubation and during recovery from anaesthesia.

The changes in heart rate variability observed in the present study reflect an increase in activity in the autonomic nervous system during physiotherapy, although the patients were sedated with propofol and alfentanil and paralysed with atracurium. Yamamura, Tomomasa and Furukawa [14] observed that halothane depressed vagal tone by up to 30% of control in cats and that this reduction was dose-dependent. The mechanism of this depression is affected by many factors [6], the most important of which is depression of the respiratory and cardiovascular centres. Most anaesthetic agents depress activity in these centres, which leads to changes in breathing pattern and cardiovascular indices. When the vagal influence on the heart is blocked or attenuated during anaesthesia, heart rate variability tends to be reduced. This variation can be recognized in both anaesthetized and sedated patients. It is likely that arousal caused by physiotherapy results in an increase in brain activity, leading to an increase in vagal tone and changes in heart rate variability.

We used circular statistical analysis to enable changes in R-R interval caused by RSA to be isolated from other causes. This is particularly useful for patients in the ICU, many of whom suffer from haemodynamic changes which cause arrhythmias in addition to RSA. In order to compare the sensitivity of linear and circular statistical analysis in detecting heart rate variability, the recordings were analysed using both methods. The results showed that circular

statistics were more appropriate than linear statistics for quantifying RSA. During physiotherapy, the changes in R , calculated using circular statistics, increased significantly, by 71%, compared with baseline values before physiotherapy. However, the mean R-R interval was only 7% shorter during physiotherapy than that observed before physiotherapy, and the change was not significant. The increase in SD from the mean ECG R-R interval during physiotherapy was 264%. This suggests that other, non-ventilatory factors had a substantial influence on the heart rate during physiotherapy. For example, the SD of the heart rate has been shown to be linked to intrinsic heart rate, affected by changes in average heart rate, and pre-ventricular contractions [15], whereas R is only minimally affected by pre-ventricular contractions and is not affected by changes in either average or intrinsic heart rate.

We also observed a small, but insignificant, change in θ during measurement of heart rate variability. θ is the mean angle of several individual ECG R-wave vectors and denotes the phase of RSA within the ventilatory cycle. During physiotherapy, θ was 8% greater than baseline and remained increased after physiotherapy. However, the changes were not statistically significant.

For use as a sedation score, the on-line trended record of RSA would be observed by ICU staff, especially preceding potentially stimulating procedures. Significant increases in R could then be followed by appropriate increases in the rate of sedative infusion. We are currently investigating methods of implementing on-line monitoring of RSA as the basis of a sedation scoring system, and the results of these studies will be the subject of further communications.

ACKNOWLEDGEMENTS

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PILOT STUDY OF AN EXPERT SYSTEM ADVISER FOR CONTROLLING GENERAL ANAESTHESIA

S. G. GREENHOW, D. A. LINKENS AND A. J. ASBURY

SUMMARY

RESAC (real time expert system for advice and control), has been developed to advise on the concentration of inhaled volatile anaesthetics during anaesthesia. It merges clinical information and on-line measurements using Bayesian inference and fuzzy logic. This paper describes a clinical trial in seven patients after initial development of the knowledge base and the human computer interface. Evaluation of the performance of RESAC included analysis of questionnaire responses. Anaesthetists were confident enough to follow the dosage advice given by RESAC in most of the patients. (Br. J. Anaesth. 1993; 71: 359–365)

KEY WORDS

Anaesthesia: control, expert systems.

Anaesthesia is a complex process in which the anaesthetist evaluates many clinical signs to form a judgement on the adequacy of anaesthesia. Using a computer to attempt this, there are two main problems: data logging, and combining and evaluating clinical signs.

It is easy to collect information automatically (e.g. heart rate and arterial pressure) from monitors such as Dinamaps. Other information (e.g. sweating and pupil diameter) can be collected only manually and suitable data entry methods are necessary. Ideally, these should require minimal keyboard use by the anaesthetist, be easy to use and should check data as they are entered.

The second problem, of combining the data, is particularly difficult because data are of different types, some are states (e.g. moving or not moving), others are continuous variables (e.g. heart rate). In addition, each of the variables has a different "degree of relevance" to the concept of "adequacy of anaesthesia". It therefore requires a logical approach completely different from that used for the assessment of one variable, often arterial pressure (SAP), as the controlled variable [1]. Other approaches have been reviewed by Chilcoat [2].

Our overall objectives were to develop an expert system which could use on-line clinical data to give an estimate of the adequacy of anaesthesia, and to use this information to provide, automatically, on-line advice about the delivery of anaesthetic agents to the patient. The overall project required a series of trials

over 3 years. Those trials provided information for the expert system knowledge database, and are not described in detail. This paper describes the last trial, comprising seven patients. In essence, we describe the performance of an on-line expert system which has had a chance to "learn" in a limited way about anaesthesia. Nevertheless, it remains relatively unsophisticated. The relevance of this paper lies largely in consideration of underlying concepts which might be used in the future.

PATIENTS AND METHODS

The study was approved by the Hospital Ethics Committee, and patients gave informed consent. Patients with various medical states were studied (in previous and present trials), so that most of the parts of the knowledge base could be developed. The main exclusion criteria related to the reliability of the clinical signs; patients were excluded if there was some pathological, physiological or pharmacological mechanism which disturbed the normal relationship between clinical signs and the usual concept of adequacy of anaesthesia. For example, the following were excluded: local anaesthetic blocks, patients taking beta-blocking and antihypertensive drugs, diabetics with neuropathy and patients with a history of stroke. Patients were excluded also if anaesthesia was likely to require knowledge beyond that available to RESAC.

Anaesthetic techniques

A variety of anaesthetic techniques (two neuromuscular block, two analgesics, spontaneous or positive pressure ventilation, etc.) was chosen deliberately for this and previous trials, to provide information on the responses to a selection of agents, in order to continue building the expert system database.

The basic pattern of anaesthesia was as follows. Premedication was with ranitidine and lorazepam orally 4 h before anaesthesia. In the anaesthetic

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room, preoxygenation, i.v. cannulation and an infusion of Hartmann's solution $7 \text{ ml kg}^{-1} \text{ h}^{-1}$ were administered. Induction was with sufficient methohexitone to suppress the eyelash reflex and tracheal intubation was facilitated with suxamethonium. The patient's lungs were ventilated with nitrous oxide, oxygen and enflurane. Manual ventilation was continued in theatre with nitrous oxide, oxygen and enflurane using a Mapleson A breathing system. Where appropriate, the patient was allowed to breathe spontaneously via the Mapleson A system.

If neuromuscular block was required for surgery, atracurium or vecuronium was given and automatic ventilation of the lungs adjusted to keep the end-tidal carbon dioxide partial pressure in the range 5.3–6.0 kPa (Normocap). A Dinamap 1846P measured systolic arterial pressure (SAP) and heart rate (HR) at 1-min intervals. ECG, tidal volume and ventilatory frequency were monitored throughout the operation. Neuromuscular block was measured by a nerve stimulator and twitch counting by palpation or a Datex Relaxograph. Blood was not required for any of the operations and Hartmann's solution was used to replace losses.

At the end of the operation, the breathing system was flushed with oxygen and any residual neuromuscular block was antagonized with neostigmine and atropine.

Recovery was evaluated by measuring time from flushing the breathing system to the patient opening their eyes and obeying commands. Anaesthetists (not the authors) were asked to evaluate the quality of anaesthesia on a five-point categorical scale (very good, good, OK, poor, very poor) on the basis of their clinical judgement, irrespective of the "opinions" of RESAC.

All patients were visited on the day after operation to check for evidence of intraoperative awareness and evidence of dissatisfaction with the anaesthetic technique.

Computer and data input

RESAC was programmed in the language "C". An Atari ST computer with 4 Mbytes of random access memory and 20-Mbyte hard disk was chosen for the laboratory development and the programs were transferred to another Atari ST with 1 Mbyte of RAM and no hard disk for operating theatre use. The theatre Atari was connected via the RS232 port to a Dinamap (1846P) and this was set to provide heart rate and arterial pressure information at 1-min intervals. When the Dinamap was not available, RESAC was programmed to receive SAP data from the keyboard. All other information was entered manually, when the patient was in the theatre. Information was requested only if RESAC needed information. Most data entry occurred during the first 10 min of the anaesthetic, when basic details such as age and sex were required.

Data entry was designed to be fast, intuitive and require minimal keyboard use. This was achieved using an indirect pointer (mouse) system with a series of screen "forms". The numeric question was the only form requiring the use of the keyboard; all others could be used with just the mouse. Whether a

particular question was asked or not, and the order of asking, in a sequence of questions, depended upon the information entered up to that point. For instance, if the SAP was considered to be irrelevant, then further questions dealing with SAP would not be asked (see reference [3] for further details of the Human-Computer Interface (HCI)).

Concepts

RESAC uses 39 data-holding variables; a representative group is listed in table I. In some cases the information has to be asked; in others the default suffices. Some of the variables would not be perceived as "information" by an anaesthetist, who would collect and use them unconsciously; RESAC, however, has to be told specifically; for example:

RESAC has to know when surgical stimulation has begun, because only then, by the internal conventions of the system, do the incoming data relate to adequacy of anaesthesia. Similarly, in the last 5 min, when the dressings are being applied RESAC has to be told to stop maintaining anaesthesia, but that the operation has not yet finished.

RESAC required data to make judgements in relation to the patient's characteristics; hence weight, age, sex, state of health, etc., were required. Doses in absolute units could then be evaluated.

RESAC had to be instructed also as to the reasonable physiological ranges for each patient and, within these, what values were ideal. For example, SAP was managed in terms of a target SAP, and an allowable variation about that target. This target and range were then used with the incoming SAP information to assign each SAP value to a band, for example below target: -15% ; within the target: $\pm 15\%$; beyond the target: $+15\%$.

TABLE I. Variables used in RESAC. The names explain the function. Variable type = class of data (e.g. defined states (nominal) or on a continuous scale (ratio)) and the range. Question form = type of screen presentation used by RESAC (a slider on a calibrated scale, a simple yes/no, a multiple choice question or numeric entry)

Name of variable	Variable type and range	Question form
AskoperationStarted	Nominal, 2 states	Yes/No
AskIfLastFiveMin	Nominal, 2 states	Yes/No
AskWeight	Ratio, 0–190	Numeric
InjectionOfMorphine	Nominal, 2 states	Yes/No
AskDosageMorphine	Ratio, 0–15	Numeric
AgeOfPatient	Ratio, 0–130	Numeric
AskTheSex	Nominal, 2 states	Yes/No
RelevanceOfSAP	Ratio	Slider
CurrentSAP (on-line)	Ratio, 0–250	None
AskTargetSAP	Ratio, 0–250	Numeric
AskRelHR	Ratio	Slider
CurrentHR (on-line)	Ratio, 0–290	None
PupilMovement	Nominal, 2 states	Yes/No
PupilSize	Ratio, 1–6	Numeric
AskRelevanceSweat	Nominal, 2 states	Yes/No
AskSweatState	Nominal, 3 states	Multiple choice
AskPatientMoving	Nominal, 3 states	Multiple choice
AskIfArtificialVent	Nominal, 2 states	Yes/No
AskRelevanceResp	Nominal, 2 states	Yes/No
TargetRespiration	Ratio, 0–50	Numeric
AskRespirationRate	Ratio, 0–60	Numeric

RESAC needed to know the extent to which something was relevant to the concept of adequacy of anaesthesia. At one extreme, this could take the form of evaluating ventilatory frequency as "irrelevant" when the patient's lungs were ventilated automatically. In another case, one might decrease the numerical "relevance" of the patient's sweating as a sign of anaesthesia because of a high room temperature.

Data output

At 1-min intervals RESAC produced a text commentary on the perceived adequacy of anaesthesia, couched in advisory terms. Warning messages were displayed only if variable boundaries were exceeded. As RESAC was tested and developed, computer code was added to give advisory comments on the concentration of enflurane based on the certainty values of the anaesthetic state hypothesis (see below). To do this, RESAC used simplified drug kinetics, considering a drug effect to be present or absent at a given instant. The anaesthetist could choose to use the advised settings or not.

Individual graphs of data (e.g. SAP) or certainty values (see below) could be displayed at will. These graphs, however, could not be updated automatically on the screen and had to be called afresh each time, for the recent information to be added. In addition to screen displays, RESAC stored all the data on disk for off-line analysis.

Knowledge base for RESAC

RESAC's knowledge base used incoming numerical facts (e.g. SAP = 120 mm Hg), and incoming probabilistic facts (e.g. Patient very ill = yes). The basic principle of the knowledge base was the use of predetermined rules to evaluate incoming facts by calculating the "certainty" associated with each of three hypotheses. The three hypotheses relating to the anaesthetic state were: "Anaesthetic: Light"; "Anaesthetic: OK"; and "Anaesthetic: Deep". Each of these was evaluated at 1-min intervals by calculating a certainty factor which had a preset range from +500 to -500, where +500 indicates "certainly true", and -500 "certainly false".

The rules are represented in the "model description language" of RESAC [4]. The system of rules is, in mathematical terms, an acyclic directed graph [5] (Appendix), which is a form of "network

of information". The network contains facts at the intersections, and rules govern movement between intersections, leading eventually to the evaluation of the anaesthetic state hypotheses in terms of certainty values.

Incoming ratio level information (e.g. SAP, HR) needs to be converted to ordinal level to use in the network. This is done by comparing the incoming value with a target value (e.g. the normal SAP of 120 mm Hg) and assigning the resulting figure to preset bands either side of the target value.

Probabilities are propagated throughout the network using Bayes theorem [6, 7] each time the current state of the knowledge base is evaluated. Fuzzy logic [8, 9] is used in some of the rules. (Bayes theorem is a statistical method for dealing with uncertain data. Fuzzy logic provides a formal, linguistic-like approach to reasoning with approximate data.)

To help in the assessment of the RESAC's own knowledge, additional off-line calculations were undertaken. These included the root mean square deviation (RMSD) of SAP and HR about their target values, and the calculation of the percentage of the operation time when a given sign was within 10% (T10%) and within 20% (T20%) of the target.

In preliminary trials, RESAC was tested in 47 patients, during which the following adjustments were made: the relative contribution of the variables to the concept of adequacy of anaesthesia; correcting serial transmission problems; digital smoothing of SAP and HR. In each case, adjustments were made off-line and RESAC was retested on more patients. (The data on these development trials are not shown here, but were given in detail in references [4] and [10].)

RESULTS

All the patients were questioned after anaesthesia and none showed any evidence of awareness.

The work detailed here comprises the study of seven patients (tables II, III). In general, the subjective ratings of this trial were satisfactory, with five anaesthetics being classed as "Good" or "Very good", and two rated as "OK".

Figures 1-6 show the certainty values for each of the three hypotheses, and the HR, SAP and recommended enflurane concentration for patients Nos 4, 5 and 7. Note that in patient No. 4,

TABLE II. Summary of clinical data. Drugs used: m = morphine; f = fentanyl; a = atracurium; v = vecuronium; n = no neuromuscular blocker used. Operation time = duration of operation as logged by RESAC

Patient No.	Sex	Age (yr)	Weight (kg)	Operation	Operation time (min)	Drugs used
1	M	67	59	Hydrocele repair	14	m + n
2	M	21	69	Excision of submandibular gland	68	m + a
3	F	44	70	Varicose veins	37	m + n
4	M	27	67	Laparotomy	48	f + a
5	F	38	66	Cholecystectomy	41	f + a
6	M	76	90	Inguinal hernia	20	m + n
7	F	41	90	Laparotomy	89	m + a

TABLE III. Summary of performance data. Quality = opinion of the anaesthetists involved in the case by clinical judgement independently of RESAC

Patient No.	Quality	Waking time (min)	Comments
1	OK	7	Patient overdosed, SAP, HR both low.
2	OK	10	Ventilatory frequency a constant 12 b.p.m. Suggested concn of enflurane supplied.
3	Good	—	During first 36.6 min suggested enflurane concn given, except for the peak of 4%. After this time RESAC suggested concns always less than 1% (0% actually given), because of timer problem for duration of op.
4	Good	5	Suggested concn supplied except for short period at 19.8 min, because of artefact in Dinamap data. Note: during whole operation the "pupils moving" sign was set to TRUE—probably a mistake.
5	Very good	5	Suggested concn supplied. At start of operation non-directed movement occurred (from 3 to 4.4 min).
6	Good	8	Suggested concn supplied throughout.
7	Very good	6	Suggested concn given only after about 13.3 min. SAP recorded as zero for part of the operation. Anaesthetic states often "ambiguous" because of conflict in evidence.
			Suggested concn supplied throughout the operation.

undergoing laparotomy (figs 1, 2), the certainty attached to the hypothesis "anaesthetic OK" reached a high level early in the operation and remained at that level, whereas in patient No. 7 also undergoing laparotomy (figs 3, 4), there were many changes of certainty.

Comparing the certainty values with the arterial pressure and heart rate data gives the impression of a situation in which a small departure from the range of reasonable values leads to a large swing in certainty values. This is shown in figures 5 and 6, where the target heart rate was 70 beat min⁻¹ and the target arterial pressure was 120 mm Hg.

In patients Nos 1, 4, 5 and 7, the anaesthetist was confident enough to use the advised concentration of enflurane, and manual adjustments were made promptly as advised by RESAC. In patients Nos 3 and 6, the anaesthetist did not use the advice of RESAC because RESAC received incorrect information from the Dinamap, and in patient No. 2 the software timer which monitored the duration of operation failed because of a software error (now specifically identified).

Table IV shows the off-line data analysis. The RMSD for HR were satisfactory, but those for SAP were larger than expected. The large RMSD for patient No. 6 was caused by an error whereby the Dinamap provided incorrect information. We can find no explanation for the large RMSD for patient No. 1.

In patients Nos 3 and 4, non-numerical clinical signs indicated that the patients were not adequately anaesthetized. In patient No. 3 the "pupils moving" clinical sign was set to TRUE for the entire operation. It is likely either that this sign was set incorrectly or that after a period of pupil movement the sign was not reset. In patient No. 4, there was non-directed movement for a short period at the start of the operation.

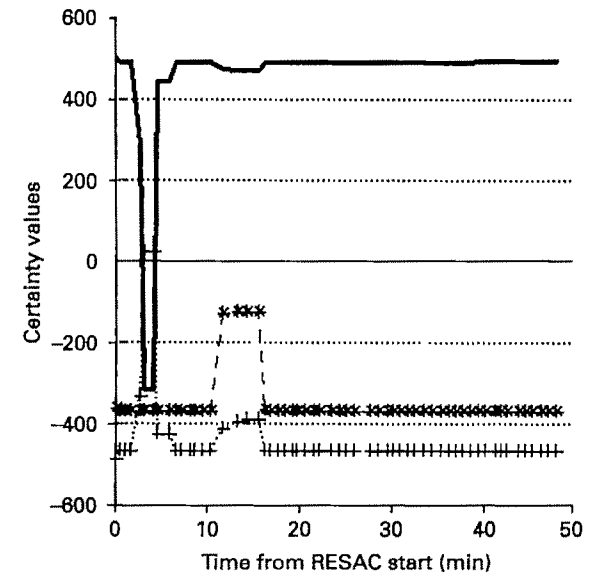


FIG. 1. Certainty values for the three hypotheses: "Anaesthetic OK" (—), "Anaesthetic Deep" (*) and "Anaesthetic Light" (+) during laparotomy in patient No. 4 (a 27-yr-old male).

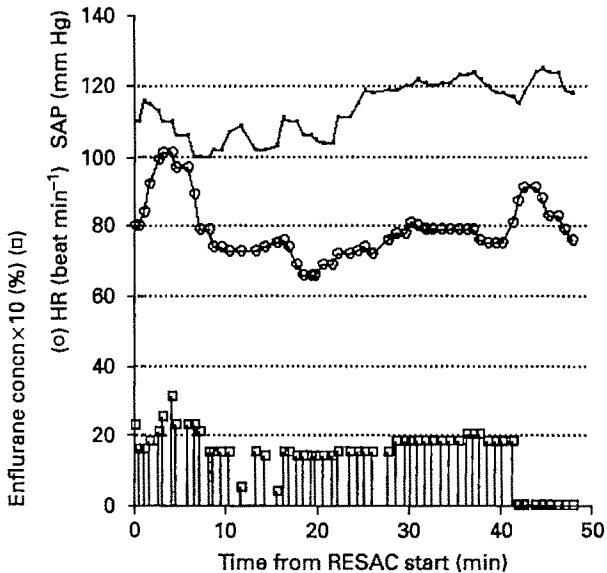


FIG. 2. Changes in HR (○), SAP (—) and recommended concentrations of enflurane (□) during anaesthesia in patient No. 4. Target SAP 121 mm Hg; target HR 75 beat min⁻¹.

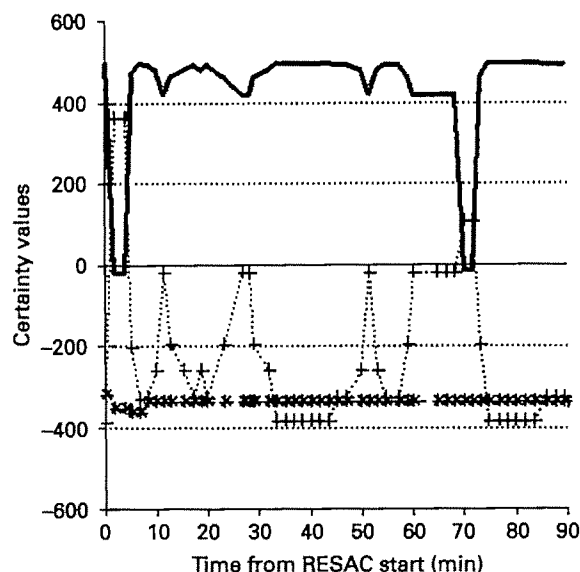


FIG. 3. Certainty values for the three hypotheses: "Anaesthetic OK" (—), "Anaesthetic Deep" (*) and "Anaesthetic Light" (+) during laparotomy in patient No. 7 (a 41-yr-old male).

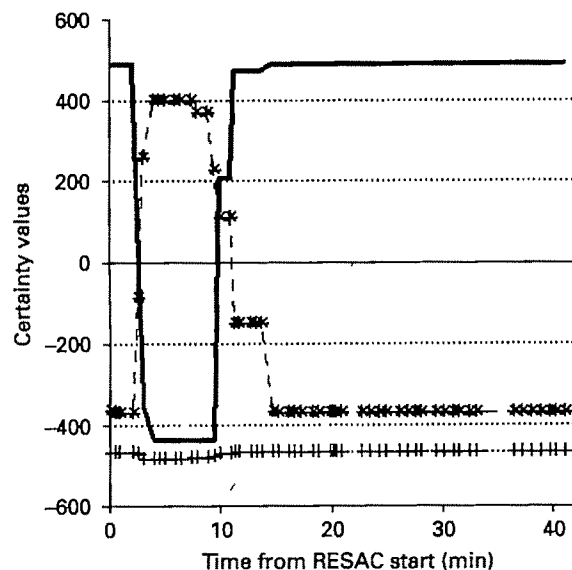


FIG. 5. Certainty values for the three hypotheses: "Anaesthetic OK" (—), "Anaesthetic Deep" (*) and "Anaesthetic Light" (+) during open cholecystectomy in patient No. 5 (a 38-yr-old female).

DISCUSSION

As judged by the initial objectives, we have the beginnings of an on-line expert system which can be used during an operation and which encapsulates some of the knowledge used by anaesthetists. When RESAC was used in practice, under the supervision of anaesthetists, the anaesthetists were confident enough to use its advice in four of seven patients, thereby effecting an advisory, on-line, closed loop control system.

Beyond the initial objectives, this study demonstrated the wide range of information constantly used by the anaesthetist. While the anaesthetist collects and processes vast amounts of information effortlessly, RESAC had to be told specifically about each minute-by-minute change. This became ob-

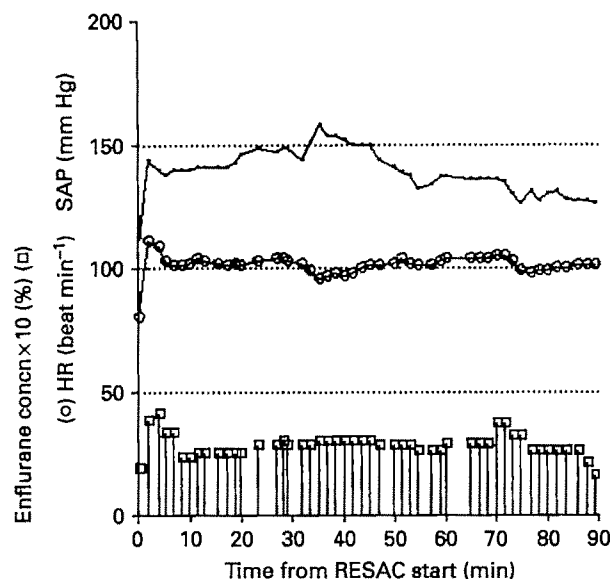


FIG. 4. Changes in HR (○), SAP (—) and recommended concentrations of enflurane (□) during anaesthesia in patient No. 7. Target SAP 128 mm Hg; target HR 80 beat min⁻¹.

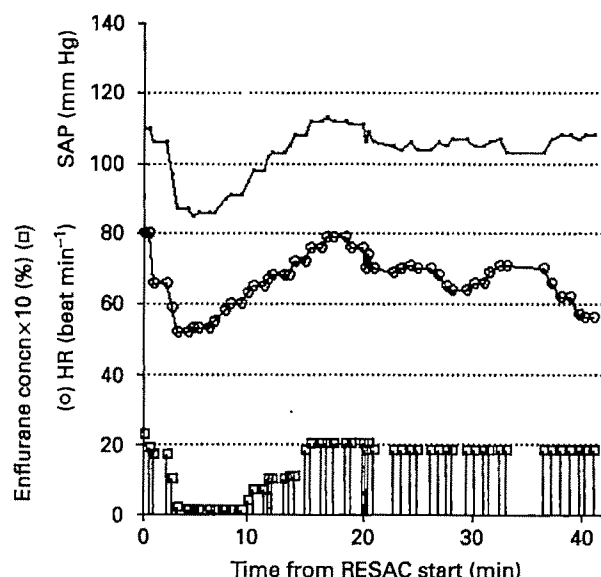


FIG. 6. Changes in HR (○), SAP (—) and recommended concentrations of enflurane (□) during anaesthesia in patient No. 5. Target SAP 120 mm Hg; target HR 70 beat min⁻¹.

vious in patient No. 3 in whom the pupils were noted to be moving; although the pupils must have stopped moving, RESAC was not told and continued to calculate with this spurious evidence of inadequate anaesthesia.

The performance of RESAC in producing adequate quality of anaesthesia seems acceptable, but the assessment of quality is inevitably subjective, and further developments will require more precise indices of adequacy of anaesthesia (e.g. evoked potentials).

The fact that the anaesthetists were content to use the enflurane concentrations advised by RESAC is difficult to interpret because it could mean anything from "this is the only and ideal dose for this patient now", to "this dose is just within what I perceive as safe and reasonable for this patient". In further

TABLE IV. Summary of on-line data for SAP and HR. RMSD = Root mean square deviation about the target value. T10% and T20% = percentages of operation time when a given sign was within 10% (T10%) and within 20% (T20%) of target

Patient No.	Mean (mm Hg or beat min ⁻¹)	Target (mm Hg or beat min ⁻¹)	T10% (%)	T20% (%)	RMSD
SAP (mm Hg)					
1	95.9	148	0	0	52.8
2	141.9	118	3	52	25.8
3	115.6	120	92	92	12.0
4	113.7	121	71	100	10.6
5	103.1	120	27	84	18.6
6	98.6	146	0	66	64.9
7	139.5	128	52	94	14.7
HR (beat min ⁻¹)					
1	64.5	80	24	30	17.4
2	81.0	70	53	76	1.3
3	78.2	80	83	92	7.9
4	79.2	75	73	86	9.4
5	66.6	70	69	90	8.3
6	68.6	80	17	91	12.4
7	101.2	80	3	4	21.6

studies, the concept of appropriate dosing needs to be defined better, although this is a complex issue incorporating both dose and time factors.

One cause of incorrect suggested enflurane concentrations was artefacts from the Dinamap. The general problem of rejecting artefacts is a major concern in any computing system which takes data from automatic monitors. The Dinamap provides a small amount of help by indicating readings which do not match its internal checks, but in most monitors such internal checking is limited to confirming that the readings lie within preset ranges. The basic problem is that, if a monitor is functioning in an unexpected way transiently (surgeon leaning on the Dinamap cuff or flushing an arterial line), the computer system has to be told specifically that the reading is an artefact.

RESAC has no ability to predict the surgical stimulus, or even the end of the operation, therefore no anticipatory action can be taken which could potentially modify an advisory response. The anaesthetist can often predict such responses and can certainly foresee the end of the operation. Such predictions imply that the anaesthetist should usually be able to achieve better recovery times than RESAC. It is unlikely that it will be possible for a machine to predict the pattern of surgical stimulation during normal surgery.

RESAC is a complex program and even so can "understand" only a limited repertoire of anaesthetic drugs, and only when they are used within a limited regimen which includes avoidance of hypovolaemia, diabetes, etc. Furthermore the "understanding" possessed by RESAC is limited to the clinical knowledge of the many anaesthetists who contributed to the knowledge base. Consequently, there are many situations which RESAC cannot address. Theoretically, the knowledge base could be expanded infinitely, but a point would soon be reached at which the knowledge would not be reliable because it dealt with rarer and smaller facets of anaesthesia, and would not be testable practically.

RESAC uses targets for some information (SAP,

HR, ventilatory frequency) and these were important because they guide the dosage strategy. To date, the target values can be based only on the experience of the anaesthetists; hence it is possible that RESAC would "perform" slightly differently for different anaesthetists.

RESAC encountered a range of patient behaviour during pilot studies and within the limits of the trials succeeded in giving sensible advice on most occasions. There are still many problems, some of which are outlined above, but the method of using an on-line expert system clearly has potential.

APPENDIX

An acyclic directed graph is a network structure consisting of a set of intersections or nodes, with interconnecting arcs. Facts are placed at the nodes. In the process of evaluating the collection of facts one moves along arcs, from node to node, in the fixed direction determined by the rules. In some cases the fact at the node can be evaluated there and then, but more commonly several other nodes have to be evaluated to produce a concept which can then be evaluated. In this manner, the whole network can be considered, leading eventually to the answering of the posed question (completing the goal).

The rules ensure that, when beginning at any arbitrary node, regardless of the path taken thereafter, return to the initial node is impossible [5]. Circular data movements must be avoided in the graph as they make the structure non-directed, which causes the program to generate computer code which cycles endlessly.

ACKNOWLEDGEMENTS

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TOWARDS A STANDARDIZED ANAESTHETIC STATE USING ISOFLURANE AND MORPHINE

H. M. ROBB, A. J. ASBURY, W. M. GRAY AND D. A. LINKENS

SUMMARY

In 34 patients undergoing major surgery, the inspired isoflurane concentration was adjusted by a control system designed to maintain systolic arterial pressure at a predetermined value. An empirical rule allowed additional morphine administration if the demand of the system for isoflurane was excessive. Satisfactory control of systolic arterial pressure was achieved in 31 patients and the anaesthetic state was clinically acceptable to an independent observer; no awareness was reported and the mean recovery time was 9.6 min. In these patients, control of systolic arterial pressure produced a pattern of clinical signs recognizable as general anaesthesia. (Br. J. Anaesth. 1993; 71: 366-369)

KEY WORDS

Anaesthesia: control of depth. Anaesthetics, volatile: isoflurane.

Despite obvious shortcomings, clinical signs are the only practical, widely accepted method of determining adequacy of anaesthesia. Although using these signs results in an acceptable anaesthetic state in most patients receiving general anaesthesia, because their interpretation is subjective there is no clinical standard of anaesthesia against which new methods of assessing anaesthetic "depth" might be compared.

We have shown that a simple control system designed to maintain systolic arterial pressure (SAP) at a predetermined value by adjusting the inspired concentration of enflurane produces a clinically acceptable state of anaesthesia [1]. This suggests that SAP is a major component in the clinical assessment of the anaesthetic state.

The objective of this study was to determine if a clinically acceptable anaesthetic state could be achieved by altering isoflurane dosage to maintain SAP at a predetermined value.

PATIENTS AND METHODS

We studied 34 ASA I and II patients admitted for elective major gynaecological surgery. Local Ethics Committee approval was obtained and all patients gave written, informed consent. Patients with cardiovascular disease and those taking drugs acting on the central nervous system (except sleeping tablets) were excluded.

Patients were premedicated with ranitidine 150 mg and temazepam 10-20 mg. Anaesthesia was in-

duced with morphine 0.1 mg kg⁻¹ and thiopentone 2.5 mg kg⁻¹. Additional thiopentone was given if the eyelid reflex was not abolished. Vecuronium 0.1 mg kg⁻¹ was given and the patient's lungs ventilated with 70% nitrous oxide in oxygen supplemented with 1% isoflurane. The trachea was intubated and the lungs ventilated using a Bain system with a fresh gas flow of 70% nitrous oxide in oxygen 100 ml kg⁻¹ min⁻¹. Carbon dioxide monitoring was not available and the fresh gas flow used was intended to maintain moderate hypocapnia [2]. Isoflurane was delivered by a Fortec 3 vaporizer (Cyprane), modified to allow it to be driven by computer control. Additional boluses of morphine were given on instruction from the program. Neuromuscular block was maintained with increments of vecuronium at 30-min intervals. The patients received Ringer's lactate solution 1 litre over the first 1 h and 500 ml every subsequent 1 h. Blood loss was replaced with extra crystalloid, colloid or blood products as indicated.

Anaesthetic adequacy was assessed clinically according to normal practice, and at 5-min intervals, using the PRST (arterial Pressure, heart Rate, Sweating, lachrymation (Tears)) score [3]. Automatic control was discontinued approximately 5 min from the end of surgery, and the nitrous oxide discontinued after the last skin suture. Any residual neuromuscular block was antagonized with neostigmine and glycopyrronium. Before tracheal extubation, patients who had not received additional morphine were given a bolus dose of 0.05 mg kg⁻¹.

Recovery was considered complete when the patient was able to respond to a request to open the eyes or protrude the tongue.

A consultant anaesthetist, not involved with the study, checked that the anaesthetic dose administered by the system was appropriate. Patients were interviewed the next day to establish if they had been aware during surgery.

During the early part of the study, three patients developed bradycardia (about 50 beat min⁻¹). These

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Correspondence to A.J.A.

incidents were managed by temporarily stopping surgery and injecting atropine. The regimen was modified so that any other patient developing a heart rate of less than 50 beat min⁻¹ during surgery would be given 0.2-mg increments of glycopyrronium until the rate was greater than 60 beat min⁻¹.

Equipment

The equipment and its inherent safety features have been described in detail elsewhere [1, 4]. In summary, a proportional-plus-integral (PI) control algorithm was implemented on a computer interfaced to a Critikon 1846 Dinamap and a vaporizer controller [4]. The vaporizer and controller were checked against a Datex Normac analyser before each case. The program was designed to maintain the patient's SAP at a target value (TSAP), which was 90% of the SAP predicted from age and sex standardized tables [5]. SAP was measured at 1-min intervals and appropriate adjustments in inspired isoflurane concentration made automatically. The vaporizer setting was determined by the following rule:

$$\text{Isoflurane concentration (\%)} = K_p e + K_i (\sum e + I_0),$$

where e = difference between the actual systolic arterial pressure and the target systolic arterial pressure (SAP-TSAP); K_p and K_i = proportional and integral gains; I_0 = a preloaded value for the integral, included to shorten the stabilization time. The gain settings used in the earlier enflurane study were retained to allow comparison [1], K_p being set to 0.1% (mm Hg)⁻¹, K_i to 0.01% (mm Hg)⁻¹ and I_0 to [120 - (age in yr)] mm Hg. The sum $\sum e$ was set to zero at the beginning of each case, and reset to zero when morphine increments were given.

Two additional rules were incorporated in the control algorithm: first, a minimum concentration of 0.6% isoflurane was delivered for the first 10 min, to avoid the risk of inadequate dosing and consequent awareness at the time of the initial surgical incision (if SAP was less than TSAP subsequent to induction); second, if the sum of five consecutive vaporizer settings exceeded 15%, the controller requested the anaesthetist to give an increment of morphine 0.05 mg kg⁻¹. In addition, the integral term was automatically reset to its preloaded value. This arrangement could be activated only at 15-min intervals. The last rule was designed to mimic the anaesthetist's response to a patient who requires large doses of volatile agent.

Statistics

The root mean square deviation (RMSD) of the SAP measurements around the TSAP was used to assess the quality of SAP control. Calculation of the RMSD is analogous to that for standard deviation, but uses the TSAP in place of the mean. Recovery times were recorded, as they could indicate a relative anaesthetic overdose, particularly towards the end of the procedure.

The requirement for additional morphine or anticholinergic agent was used to classify the patients into three groups, as described in the results section.

TABLE I. The surgery performed. Group A received neither additional morphine nor an anticholinergic agent. Group B received additional morphine alone and group C an anticholinergic agent alone

Operation	Group		
	A	B	C
Hysterectomy and salpingo-oophorectomy	13	3	8
Ovarian cystectomy	2	—	1
Vaginal hysterectomy, pelvic floor repair	1	—	1
Reversal of sterilization		1	
Bilateral salpingo-oophorectomy		1	

Statistical analysis was carried out with the Statmode package (v 1.05; SC Coleman) on an Amstrad PC1640 computer, running under MSDOS v 3.02 (Microsoft). Intergroup comparisons were made with a Mann-Whitney U test (two-tailed); chi-square with Yates' correction was used to assess the association between sweating and administration of anticholinergic agent. $P < 0.05$ was considered significant.

RESULTS

Details of the procedures are listed in table I and an example of the progress of automatic control is shown in figure 1. Adequate control of SAP was not achieved in one patient, despite additional morphine, and a breach of procedure occurred in two others: one did not receive an increment of morphine on time and the second suffered a sudden 3.5-litre blood loss, resulting in an SAP less than TSAP for 50 min. Results from these three patients were excluded from analysis.

The remaining patients were classified into groups on the basis of their responses under anaesthesia with the control system. Group A (16 patients) received morphine at induction only, while group B (five patients) required one additional dose of morphine. No patient required more than one dose of morphine. Group C (10 patients) received an anticholinergic agent to treat bradycardia. No patient who received an anticholinergic agent required additional morphine.

No patient complained of awareness when questioned after operation. The anaesthetic state was acceptable to the supervising independent anaesthetist in all cases. This is reflected by the mean PRST score of 0.9. In addition, SAP (mean 118.2 (SD 6.6) mm Hg), heart rate (mean 78.3 (10.5) beat min⁻¹) and rate-pressure product (mean 9246 (1326) mm Hg min⁻¹) were acceptable. The mean recovery time was 9.7 (7.8) min. All patients indicated that they would accept the same anaesthetic again.

Table II summarizes the data from the three groups. There was no significant difference between the three groups in age, weight, arterial pressure before induction of anaesthesia, TSAP or total time from induction to discontinuation of the nitrous oxide. The means of the individual RMSD values for all groups were small and there was no significant difference between groups. The mean difference between TSAP and SAP was significantly greater in group B compared with that in the two other groups. Mean heart rate, rate-pressure product and inspired

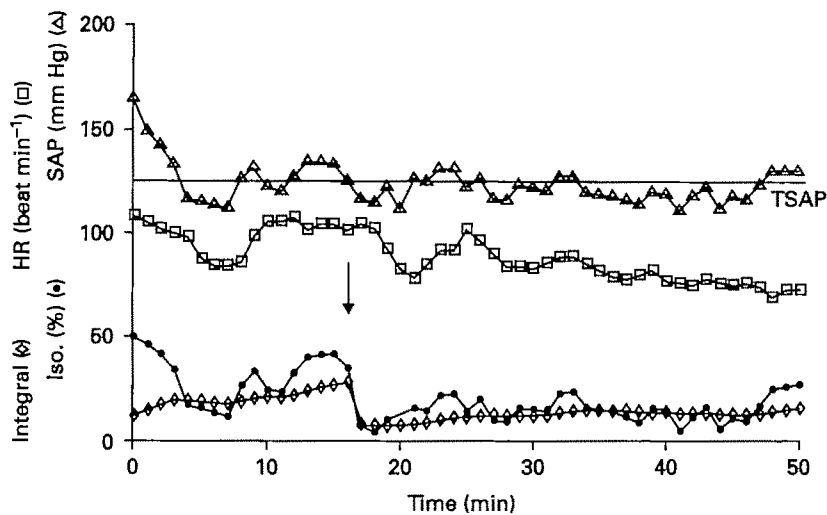


FIG. 1. Example of the control achieved in a 50-yr-old patient undergoing an abdominal hysterectomy who was given additional morphine at the time indicated by the arrow. RMSD = 7.46 mm Hg. HR = Heart rate; SAP = systolic arterial pressure; Iso. = inspired isoflurane concentration. TSAP = target SAP.

TABLE II. Details of anaesthesia and cardiovascular variables in the three groups (mean (range)). TAT = Total anaesthesia time; ATPR = time from end of anaesthetic to patient response; TSAP = target systolic arterial pressure; SAP = mean measured systolic arterial pressure; RMSD = root mean square deviation; HR = mean heart rate; Isoflurane = mean inspired isoflurane concentration; RPP = rate-pressure product; PRST = PRST score. Significant difference ($P < 0.05$) compared with: * group A; † group C

	Group A (n = 16)	Group B (n = 5)	Group C (n = 10)
Age (yr)	43.1 (32-65)	37.6 (26-73)	42.2 (33-62)
Weight (kg)	60.1 (48-82)	64.6 (45-91)	64.3 (53-82)
TAT (min)	65 (34-106)	69.8 (58-92)	62.3 (58-96)
ATPR (min)	7.1 (1-21)	20.4 (13-36)*†	8.5 (2-22)
TSAP (mm Hg)	118.0 (110-133)	114.4 (106-118)	117.3 (111-131)
SAP (mm Hg)	118.4 (109-133)	119.0 (110-124)	117.6 (112-132)
RMSD (mm Hg)	7.0 (4.2-11.7)	8.5 (4.8-10.9)	7.7 (5.8-10.6)
HR (beat min ⁻¹)	74 (63-87)	89 (77-99)*	80 (64-99)
Isoflurane (%)	1.0 (0.3-2.0)	2.1 (1.8-2.5)*†	0.8 (0.1-1.7)
RPP	8677 (7192-11100)	10634 (9603-12248)*	9462 (6788-11452)
PRST	0.82	1.10	0.95

isoflurane concentration were significantly smaller in group A than in group B. Of these, only the required dose of isoflurane was greater in group B than in group C. There was no difference in heart rate, rate-pressure product or isoflurane requirement between groups A and C. The patients in group B took significantly longer to recover than those in the other groups.

Sweating was not observed in those patients who received an anticholinergic agent, but was observed in the two other groups. The association between sweating and administration of anticholinergic agent was statistically significant.

DISCUSSION

The control system achieved the objective of maintaining systolic arterial pressure at its target and this resulted in a clinically acceptable pattern of clinical signs, supporting our view that SAP is a major component of the clinical assessment of the anaesthetic state.

The differences between groups A and B may be explained by the rule that gave rise to the additional morphine doses. The greater isoflurane (and mor-

phine) requirement in group B is an obvious example. Other differences such as the greater heart rate in group B may reflect either increased autonomic activity or an effect related to the larger doses of isoflurane these patients received. Despite these differences, anaesthesia was clinically acceptable in both groups.

The only significant difference between groups A and C was the greater heart rate in group C. Otherwise, groups A and C were similar. Moderate doses of anticholinergic agents did not appear to detract from the usefulness of SAP as an indicator of anaesthetic adequacy. Further studies, in which some patients would receive an anticholinergic agent at induction of anaesthesia, are required to confirm this finding.

No patient in our previous study with enflurane had bradycardia sufficient to require treatment [1], but three patients in this study required rapid treatment for bradycardia. We do not know why bradycardia might occur more frequently in patients anaesthetized with isoflurane, but it may be simply a surgical effect of peritoneal traction.

The PRST score is a simple method of measuring the clinical adequacy of anaesthesia [3]. The greater

mean PRST scores in those patients who required morphine or received an anticholinergic agent reflected the greater heart rates in these groups. This probably represents a pharmacological effect rather than an increase in autonomic activity.

Our observation that sweating was absent in patients who received anticholinergic drugs is not surprising, and suggests that anticholinergic agents might limit the usefulness of sweating as a sign of inadequate anaesthesia. The PRST score, which includes a score for sweating, must be interpreted with care under these circumstances.

Although the use of clinical signs (SAP in this case) to guide anaesthetic dosage may be criticized [6, 7], these signs are used widely in current anaesthetic practice and it is therefore highly likely that they carry useful information. Obviously, the usefulness of clinical signs may be reduced by drugs such as beta-blockers, conditions such as hypertension or intraoperative "events" such as surgical haemorrhage. The system we have described would be of limited routine clinical use in these situations. However, a standardized anaesthetic state, used under controlled circumstances, would be useful in evaluating the newer techniques (such as monitoring evoked potentials) proposed to assess the anaesthetic state [8].

As anaesthetists use combinations of clinical signs and anaesthetic agents to achieve an anaesthetic state, our single input (SAP), single output (isoflurane) system has obvious limitations. However, if the control system can be developed to use additional clinical signs, the clinical anaesthetic state will be defined objectively by the algorithm on which the system is based. Several factors must be considered before addition of other clinical signs: many signs (e.g. tears) are subjective; the measurement of some is difficult to automate (e.g. pupil diameter [9]); the value of others might be limited (e.g. sweating); finally, weighting must be applied to each additional sign.

We chose SAP as a starting point as it is an easily measured objective sign with a well established range of acceptable values. In our system, the target systolic pressure (TSAP) effectively set the depth of anaesthesia. The choice of TSAP was difficult, as single measurements in the ward or anaesthetic room are likely to be unrepresentative of the patient's true resting values because of anxiety or sedation [10]. Our use of tables to determine TSAP overcame the immediate problem, but suffered the disadvantage that it had a population rather than an individual basis. The requirement for additional morphine in our studies probably reflected an inappropriately small TSAP for individual patients rather than a relative resistance to anaesthetic agents; this in-

terpretation is supported by the significantly longer recovery times in those patients who required additional morphine. Repeated measurements in the ward using a non-invasive arterial pressure monitor may give a more realistic value for the TSAP.

A major limitation of control systems is that they cannot predict changes in external circumstances, such as surgical stimulation. In addition, our system was originally designed to control SAP with a single agent, whereas anaesthetists tend to limit the requirement of a volatile agent by use of opioids. We attempted to overcome these problems by the incorporation of the additional rules. As a few patients took a prolonged time to recover from the anaesthetic, despite the additional rules, these may require modification if an individually determined value of TSAP does not overcome this problem.

Building on the pioneering work of Suppan [11], our studies have laid some groundwork for developing a more flexible control system which may mimic more closely the activity of the anaesthetist. We believe that the system requires more patient information in order to give reliable control in a wider range of conditions, and plan to develop it to include additional clinical signs as additional controlled variables.

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NEW PATCH DELIVERY SYSTEM FOR PERCUTANEOUS LOCAL ANAESTHESIA

D. F. McCAFFERTY AND A. D. WOOLFSON

SUMMARY

We have assessed the release of amethocaine from a new patch delivery system and subsequent drug diffusion through human stratum corneum and whole skin. We found that the patch system was more efficient than an amethocaine gel preparation. It was also observed, both in vitro and in vivo, that the stratum corneum acted as a reservoir for amethocaine. A double-blinded clinical trial, using 30- and 60-min application times, indicated that there was no statistical difference between patch and gel formulations in onset of percutaneous local anaesthesia. Furthermore, a 30-min application of the patch was sufficient to provide profound and prolonged topical anaesthesia in all volunteers. In contrast, although a 60-min application of EMLA was necessary to ensure satisfactory onset of percutaneous anaesthesia, the duration of action was much shorter than that of the amethocaine patch. (Br. J. Anaesth. 1993; 71: 370-374).

KEY WORDS

Anaesthetics, local; amethocaine, EMLA.

The most common form of medically induced pain is that caused by the insertion of needles, for example for venepuncture. It has been reported that one of the major fears of young children in hospital concerns procedures involving needles [1, 2]. Therefore, a rapidly acting topical anaesthetic formulation would be of considerable clinical benefit in reducing the fear, anxiety and pain associated with invasive medical procedures.

Although EMLA, a topical percutaneous anaesthetic gel consisting of prilocaine and lignocaine, provides satisfactory skin anaesthesia, the minimum recommended time of application to ensure adequate anaesthesia is 1 h. The preparation is applied as a thick layer under an occlusive dressing and duration of anaesthetic effect is approximately 30 min. A further problem associated with any cream- or gel-based topical preparations is that the amount of drug applied topically, and area of skin surface involved, are ill-defined.

It has been reported that a percutaneous local anaesthetic gel formulation containing amethocaine base provided more rapid and prolonged anaesthesia than EMLA [3]. The anaesthetic efficacy of this formulation derives partially from the solid-to-liquid phase change which occurs when amethocaine base, in an aqueous environment, is exposed to temper-

atures of about 29 °C and greater [4]. We have now further developed this amethocaine phase-change system [5] to produce a percutaneous local anaesthetic patch, similar in appearance to many existing typical transdermal therapeutic systems, which allows enhanced shelf-life, greater convenience of use and the precise control of the dose of drug administered. The present study reports on the *in vitro* penetration characteristics of amethocaine from the new patch system and compares its clinical efficacy against EMLA in a double-blind, placebo-controlled volunteer study.

MATERIALS AND METHODS

Chemicals

Amethocaine base U.S.P. was obtained from Orgamol, Switzerland. EMLA was obtained from Astra Pharmaceuticals Ltd, Hertfordshire, U.K. Trypsin type 11: crude was obtained from Sigma Ltd, Poole, Dorset. All other chemicals were of Analar or HPLC quality.

Formulations

The percutaneous local anaesthetic patches (3 × 3 cm) contained amethocaine 10 mg and were prepared using conventional pharmaceutical methods [6]. The amethocaine gel formulation was prepared as described previously [3]. Controls, without amethocaine, were similarly prepared. EMLA was repacked into plain tubes and a matching placebo cream was prepared also.

Diffusion studies

In vitro penetration of amethocaine through human skin was undertaken using modified Franz diffusion cells (FDC-400, flat flange, 15-mm orifice diameter, Crown Glass Co., Somerville, NJ, U.S.A.), as described previously [7]. All experiments were conducted in triplicate. Flux values were calculated by linear regression from the linear sections of the concentration per unit area *vs* time plots.

Amethocaine assay

Amethocaine was assayed by ion-pair reverse phase high pressure liquid chromatography using a

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Spherisorb 5 mm ODS column (25 cm). The mobile phase consisted of acetonitrile:water (8:2) containing 1-hexanesulphonic acid ion pair reagent $0.005 \text{ mol litre}^{-1}$ (Waters Ltd, Harrow, U.K.). A Gilson 302 pump was used in combination with an 802 manometric module (Anachem Ltd, Luton, U.K.) and a Rheodyne 7125 injector (20- μl loop). Detection was carried out at 310 nm using an LKB 2151 variable-wavelength detector (LKB, Bromma, Sweden). Chromatograms were recorded on a Hewlett-Packard 3390A integrator (Hewlett-Packard, Wokingham, U.K.). Calibration graphs were linear ($r > 0.999$) in the range $6.5\text{--}55 \mu\text{g ml}^{-1}$. Procaine hydrochloride was used as the internal standard.

Preparation of human whole skin samples

Healthy skin samples, surplus to limb amputation, were obtained from the Belfast City Hospital. Human whole skin samples were prepared by careful removal of the subcutaneous fat. Skin sections were cut of a size suitable for mounting in the diffusion cells. Prepared skin samples were stored frozen at -18°C until required.

Preparation of human stratum corneum

Human whole skin was incubated in trypsin solution 0.1% w/v in phosphate-buffered saline, pH 7.2, at 37°C . The stratum corneum was then peeled carefully from the epidermis, rinsed in water and cut into sections of suitable size.

In vitro studies

The Franz diffusion cell apparatus was configured as previously described [7]. Each drug penetration experiment was performed simultaneously in triplicate using whole skin or stratum corneum samples from the same source. The receiving chamber of each cell was filled with phosphate buffered saline (12 ml, pH 7.2) and allowed to equilibrate for 1 h. Skin samples were placed on a filter support and covered with either samples of the patch or a thin layer (1 mm) of gel, using a casting bar technique. They were then covered with an occlusive membrane (Parafilm). The receiving fluid was stirred continuously and synchronously in all three cells. Samples (0.3 ml) were obtained at intervals, amethocaine concentration was measured and the reservoir topped up with 0.3 ml of phosphate buffered saline. Previous studies have indicated that the amount of amethocaine metabolized during the course of the experiments would be negligible [8].

Volunteer studies

After obtaining Ethics Committee approval, we studied 20 healthy, trained volunteers (12 female), age range 19–32 yr. Each volunteer gave written informed consent. The purpose of the *in vivo* study was to compare the speed of onset of anaesthesia, effectiveness and duration of action of the patch delivery system with the gel preparation and EMLA. Because of difficulties with double-blinding this part of the study (there are marked physical differences between the patch and the gel formulations), appropriate placebos were also included for each

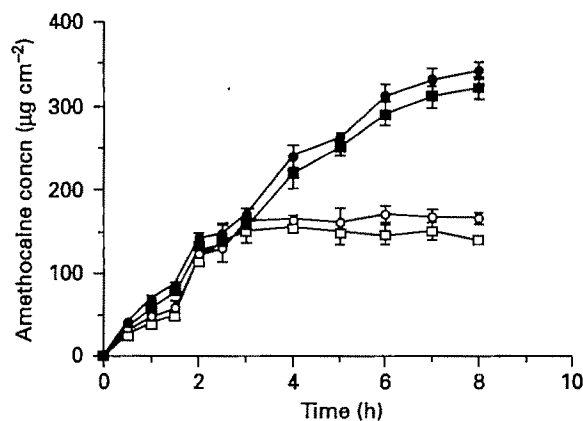


FIG. 1. Penetration of amethocaine through human stratum corneum after 30-min and continuous application of a gel and patch formulation. □ = Gel, 30 min; ○ = patch, 30 min; ■ = gel, continuous; ● = patch, continuous.

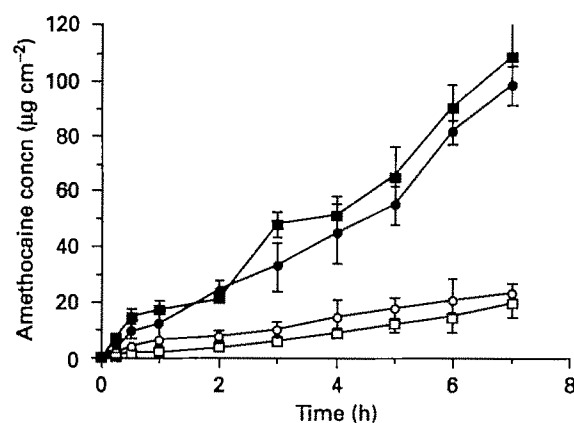


FIG. 2. Penetration of amethocaine through human whole skin after 30-min and continuous application of a gel and patch formulation. □ = Gel, 30 min; ○ = patch, 30 min; ■ = gel, continuous; ● = patch, continuous.

preparation. Therefore, each volunteer was tested with six preparations, three active and three placebos, in a randomized manner. A minimum period of 7 days elapsed between treatments. The formulations were applied to the ventral surface of the forearm, as described previously [3]. Gel 1.0 g, patch and EMLA 1.0 g were left in contact with the skin for both 30- and 60-min periods of application. After removal of the preparations, the treated sites were wiped clean and anaesthesia was assessed by pin-prick as described previously [9, 10]. Full anaesthesia was allotted a score of 1, partial or no anaesthesia was scored as 0 [3].

RESULTS

The diffusion of amethocaine through human stratum corneum is shown in figure 1 and that through human whole skin from both formulations, under the same conditions, in figure 2. The flux values obtained from these data are presented in table I. Application of ANOVA (single factor analysis of variance with repeated measures) to the data indicated that there were significant differences between the flux values, therefore the Scheffé multiple comparison test was applied to obtain more

TABLE I. Flux values for gel and patch through human membranes (mean (SD))

Membrane	Flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$)
Gel: 30-min application	
Human stratum corneum	49.74 (2.11)
Human whole skin	2.13 (0.17)
Gel: continuous application	
Human stratum corneum	58.18 (1.34)
Human whole skin	14.67 (0.64)
Patch: 30-min application	
Human stratum corneum	53.89 (1.13)
Human whole skin	2.31 (0.15)
Patch: continuous application	
Human stratum corneum	64.83 (2.17)
Human whole skin	18.47 (1.41)

TABLE II. Comparison of amethocaine fluxes of gel and patch using human stratum corneum for two different application times (Scheffé multiple comparison test). c = Continuous application; 30 = 30-min application

Formulation/application time	Significance of difference
Patch (c) vs gel (c)	$P > 0.05$
Patch (c) vs patch (30)	$0.001 < P < 0.05$
Patch (c) vs gel (30)	$0.001 < P < 0.05$
Gel (c) vs patch (30)	$P > 0.05$
Gel (c) vs gel (30)	$P > 0.05$
Gel (30) vs patch (30)	$P > 0.05$

TABLE III. Comparison of amethocaine fluxes of gel and patch using human whole skin for two different application times (Scheffé multiple comparison test). c = Continuous application; 30 = 30-min application

Formulation/application time	Significance of difference
Patch (c) vs gel (c)	$P > 0.05$
Patch (c) vs patch (30)	$0.001 < P < 0.05$
Patch (c) vs gel (30)	$0.001 < P < 0.05$
Gel (c) vs patch (30)	$0.001 < P < 0.05$
Gel (c) vs gel (30)	$0.001 < P < 0.05$
Gel (30) vs patch (30)	$P > 0.05$

TABLE IV. Comparison of amethocaine fluxes of gel and patch through human stratum corneum (sc) and whole skin (ws) for two different application times (Scheffé multiple comparison test)

Formulation/barrier membrane	Significance of difference
30-min application	
Gel (sc) vs gel (ws)	$0.001 < P < 0.05$
Patch (sc) vs patch (ws)	$0.001 < P < 0.05$
Gel (sc) vs patch (ws)	$0.001 < P < 0.05$
Patch (sc) vs gel (ws)	$0.001 < P < 0.05$
Continuous application	
Gel (sc) vs gel (ws)	$0.001 < P < 0.05$
Patch (sc) vs patch (ws)	$0.001 < P < 0.05$
Gel (sc) vs patch (ws)	$0.001 < P < 0.05$
Patch (sc) vs gel (ws)	$0.001 < P < 0.05$

detailed comparisons. Statistical comparisons of drug fluxes through human stratum corneum and human whole skin are shown in tables II and III, respectively. Table IV gives statistical comparisons of the drug flux values obtained for both the gel and patch formulations through each of the two barrier membranes.

It is evident from table II that, with two exceptions, the use of different application times had

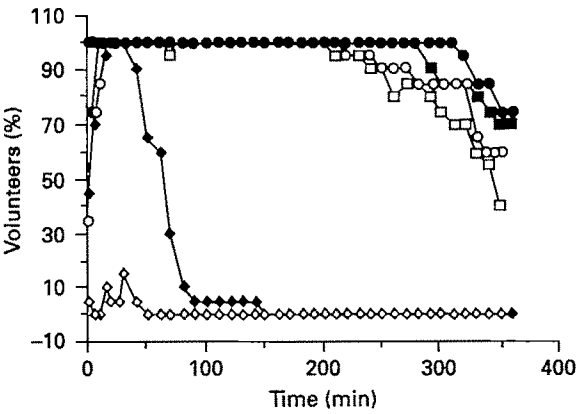


FIG. 3. Percentage volunteers anaesthetized after either 30- or 60-min applications of an amethocaine gel and patch formulation and EMLA. Time 0 = Removal of formulations from skin. $\square$ = Gel, 30 min; $\circ$ = patch, 30 min; $\diamond$ = EMLA, 30 min; $\blacksquare$ = gel, 60 min; $\bullet$ = patch, 60 min; $\blacklozenge$ = EMLA, 60 min.

little effect on the flux values of amethocaine through human stratum corneum, for both gel and patch formulations. It was also observed that the type of formulation used had no significant effect ($P > 0.05$) on the flux values. The flux of amethocaine obtained when the patch was applied continuously was significantly better ($0.001 < P < 0.05$) than that provided when either the patch or gel was applied for 30 min.

Although the type of amethocaine formulation used had no significant effect ($P > 0.05$) on the drug flux through human whole skin, time of application (table III) did prove significant ($0.001 < P < 0.05$). The type of barrier membrane used significantly ($0.001 < P < 0.05$) affected the flux values obtained for both application times (table IV).

Initially, the patch formulation was tested for percutaneous anaesthetic efficacy against placebo in a double-blind design. Statistical analysis of the results (chi-square) indicated that it was significantly ($P < 0.001$) better than placebo. All the other formulations used in this study also provided significantly ($P < 0.001$) better percutaneous local anaesthesia compared with placebo.

The *in vivo* efficacy of the gel, patch and EMLA, following both 30- and 60-min periods of application is shown in figure 3. For the 30-min application time, only the speed of onset of the gel and patch formulations were compared (table V), as EMLA provided anaesthesia only in a small number of volunteers (fig. 3). The mean time for onset of skin anaesthesia was 35.3 min for the gel formulation and 34.5 min for the patch. For the 60-min application, however, the three different formulations were compared statistically for speed of onset of anaesthesia, against one another. Application of ANOVA to the data indicated that there was a significant difference between the formulations. Therefore, the Scheffé multiple comparison test was used to obtain more detailed comparisons (table V). The results indicated that both the gel and patch were significantly more rapid than EMLA in providing percutaneous local anaesthesia ($P < 0.001$) after a 60-min application. In contrast, there was no

TABLE V. Comparison of speed of onset of percutaneous local anaesthesia for various formulations. **t* test; †Scheffé multiple comparison test

Anaesthetic formulation	P
30-min application	
Gel <i>vs</i> patch*	> 0.1
60-min application	
Gel <i>vs</i> patch†	> 0.5
Gel <i>vs</i> EMLA†	< 0.001
Patch <i>vs</i> EMLA†	< 0.001

significant difference between the gel and the patch formulations in onset of anaesthesia (table V). All volunteers were anaesthetized after the 60-min application when either the gel or patch was used, but this was not the case with EMLA (fig. 3), for which the mean time of onset of percutaneous local anaesthesia was 65.3 min.

DISCUSSION

The potential benefits of a patch delivery system for topical percutaneous local anaesthesia are increased safety and convenience of use, reduced drug-loading and application of an accurately known amount of drug onto a clearly defined area of skin. However, because of the different formulation characteristics of a patch in comparison with gel, it was necessary, initially, to examine the release behaviour of amethocaine from the patch in respect of *in vitro* skin penetration characteristics. It can be observed clearly (figs 1, 2) that diffusion of amethocaine occurred from the patch through both human stratum corneum and whole skin. Not surprisingly, the amount of drug penetrating human whole skin was substantially less than that diffusing across human stratum corneum. Although this difference could be attributed to the greater thickness of whole skin, it was likely that increased protein binding was also reducing penetration of drug [8]. It can be seen also that the patch provided similar flux values through both membranes compared with gel (table I), despite the total amount of amethocaine applied to the skin samples in the form of the patch being about 50% that of the gel. This apparent anomaly may be explained by considering the diffusion of amethocaine from the formulations. Effectively, amethocaine penetration into the skin, with either patch or gel formulations, occurs from a saturated drug solution at the formulation-skin interface, the diffusion layer [5]. As drug is depleted from this interface, it is constantly replaced by undissolved amethocaine. Therefore, it would require prolonged application of either formulation in order to deplete completely the available amethocaine in the diffusion layer—a phenomenon recognized with other topical formulations [11]. Therefore, as the hydrated patch simulates a very thin layer of gel, it provides a comparatively efficient method of drug delivery to the skin surface.

If amethocaine diffusion across human stratum corneum is considered after a 30-min application time, it can be observed that drug continues to appear in the receiving fluid even after the patch is removed, illustrating the reservoir effect of this

tissue. The amount of drug diffusion across stratum corneum stopped after about 2 h, giving a final value of $155 \mu\text{g cm}^{-2}$. An approximate estimate of the amount of amethocaine contained, as a reservoir, within 1 cm^2 of stratum corneum can be obtained by considering the difference in the amount of drug in the receptor after 30 min compared with that observed when a plateau occurred; this gives a value of about $125 \mu\text{g}$.

These data would indicate that about 1.4 mg of drug could diffuse from a $3 \times 3 \text{ cm}$ patch, which initially contained amethocaine 10 mg. Even when the patch was left on continuously for a 7-h period, the amount of amethocaine diffusing through was about $350 \mu\text{g cm}^{-2}$. This represents diffusion of approximately 3.2 mg of drug from a similar sized patch. However, it is interesting to observe that, in the latter case, the diffusion rate began to plateau after this prolonged period of time. At this point the drug concentration in the diffusion layer at the skin surface was becoming depleted and the rate of absorption was reduced. This phenomenon provides a potential safety feature, in that an application time extending to several hours would be required to exhaust the patch of drug. It must be emphasized that the projected amounts of amethocaine penetrating the skin, as discussed above, represent an extreme case. Only penetration of the stratum corneum is being considered and amethocaine metabolism, both within the skin and systemically, is ignored [12]. It has been shown [13] that, after application of 5% amethocaine 2 g to a 6-cm^2 area on the dorsum of the hand for a 4-h period in 10 adult volunteers, amethocaine could be detected in only three subjects. The maximum measured plasma concentration was 200 ng ml^{-1} . Although amethocaine, *in vivo*, is metabolized rapidly to p-(butyl-amino)benzoic acid [8], this metabolite could only be detected at a maximum concentration of 820 ng ml^{-1} in one volunteer [13].

In vivo studies on the activity of patch compared with placebo clearly indicated that the former provided effective local anaesthesia similar to that of the gel. Both formulations provided satisfactory anaesthesia in all volunteers (fig. 3), although it can be seen that some volunteers were already anaesthetized on removal of both patch and gel formulations after the 30-min application period. It has been shown that a minimum application period of approximately 20 min is necessary to provide percutaneous local anaesthesia [14]. All volunteers were found to be anaesthetized within 15 min after removal of the amethocaine formulations (fig. 3). This delayed action effect can be related to the results from *in vitro* drug penetration studies, in which amethocaine continued to diffuse into the receiving fluid even after the preparation had been removed from the skin samples (fig. 1). Therefore, a reservoir effect is apparent both *in vivo* and *in vitro*. This phenomenon has been well documented after the topical application of various steroids, although the reservoir effect with steroids is much more pronounced than that with amethocaine.

It is clear from figure 3 that EMLA does not produce adequate percutaneous local anaesthesia

after a 30-min application. Although a small number of volunteers were anaesthetized, the effect was of short duration (fig. 3). This is in agreement with previous observations [3]. Both lignocaine and prilocaine, the constituent local anaesthetics of EMLA, are less potent than amethocaine and hence would be required in larger concentrations than the latter drug to produce similar levels of local anaesthesia.

When the time of application was increased to 60 min, there was a marked improvement with the anaesthetic effect of EMLA (fig. 3). Although the increased time of application had a less dramatic effect on the behaviour of the amethocaine formulations, some differences were noted; for example, all volunteers were found to be anaesthetized on removal of the formulations (fig. 3). It would appear also that an increase in application time tended to cause a concomitant increase in the duration of the anaesthetic effect (fig. 3). There is a marked difference in the duration of action between EMLA and both amethocaine formulations. Thus, even with an application time of 30 min, using the latter formulations, the shortest period during which all volunteers were satisfactorily anaesthetized was just over 3 h. However, it can be observed (fig. 3) that some volunteers had an anaesthetic effect extending beyond 6 h. In contrast, a 60-min application of EMLA resulted in all volunteers being anaesthetized for a minimum of 20 min (fig. 3), although some individual volunteers had satisfactory anaesthesia of much longer duration. Although penetration enhancers have been used in an attempt to increase the rate of diffusion of amethocaine through skin, and hence reduce the time required for onset of anaesthesia, these are unlikely to have a clinically significant effect [15]. A liposomal percutaneous anaesthetic formulation containing 0.5% w/w amethocaine has been shown to produce satisfactory topical anaesthesia [16]. However, when an application time of 30 min was used, a further period of 1.5 h was required for onset of anaesthesia in 80% of the volunteers. This prolonged time for onset of effect is, perhaps, not surprising, considering the design of the formulation: amethocaine is trapped within vesicles from which it must escape before penetrating the skin.

In the present study, only one application site was used as it is well established that various sites of the body provide different challenges to percutaneous penetration. For example, drug penetration is easier through the scalp (or the skin behind the ears), whereas it is more difficult through the palms of the hands or soles of the feet. The main sites for application of percutaneous local anaesthetic formulations would be the ventral surface of the forearm or the dorsum of the hand, for venepuncture. Although it has been suggested that the dorsum of the hand provides a greater barrier to local anaesthetics, in

terms of onset of anaesthesia, this does not appear to be a problem when amethocaine is used, particularly in children [17].

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DERMAL ANAESTHESIA: COMPARISON OF EMLA CREAM WITH IONTOPHORETIC LOCAL ANAESTHESIA

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SUMMARY

We have compared the efficacy of two non-invasive methods of transdermal anaesthesia: application of EMLA cream and iontophoresis of 5% lignocaine with adrenaline 1:50 000 in six healthy subjects. We tested depth of tissue penetration (pinprick) and effect on pain evoked by i.v. injection. After iontophoresis, pain on i.v. injection was abolished in five of six volunteers, whereas EMLA had no effect. We conclude that local anaesthetics penetrate deeply enough to numb both veins and skin with iontophoresis only. (Br. J. Anaesth. 1993; 71: 375–378)

KEY WORDS

Anaesthesia, local: iontophoresis; EMLA.

Pain and discomfort associated with insertion of i.v. needles have been shown to be reduced significantly by EMLA (eutectic mixture of local anaesthetics) cream after topical application [1, 2]. However, there has been little attention paid to its ability to prevent pain on i.v. injection of several drugs used in anaesthesia [3]. Iontophoresis of local anaesthetics enhances transdermal ionic transport [4, 5]. Although effective in reducing the pain of needle insertion [6], it has never been compared with EMLA cream for either venepuncture or i.v. administration of pain evoking drugs. This is surprising, since iontophoretically applied local anaesthetics are effective within 10 min [6–8], whereas EMLA cream requires a rather long application time of up to 1 h [9–11]. We have therefore compared the efficacy of EMLA cream with that of iontophoretically applied local anaesthetic on pain during venepuncture and during i.v. injections.

SUBJECTS AND METHODS

After approval by the Ethics Committee of the University of Düsseldorf and informed consent had been obtained, we studied six healthy volunteers (five male; ages 27–30 yr). Experiments started at 15:00 with the subject sitting comfortably, semi-recumbent at an ambient temperature of 20–22 °C.

Experimental design

Effects of each method of application were tested simultaneously on the ventral surface of the forearms of each volunteer. Two areas of about 20 cm² were

selected so that a large vein crossed the full length of the area (fig. 1).

Pain during venepuncture was induced by inserting a 27-gauge cannula. To assess drug evoked pain (i.e. to test sensory innervation of the vein) 2 ml of hyperosmolar saline (2500 mosmol kg⁻¹, pH 7.4, temperature 24 °C) was injected within 10 s while the vein was compressed proximally for 1 min; saline 2500 mosmol kg⁻¹ (an osmolality more than 8-fold that of blood) has been demonstrated always to evoke pain in isolated vein segments [3]. To determine depth of tissue penetration provided by each method of application, a 27-gauge cannula was inserted perpendicularly through the skin in five well-defined sites within the numbed area until pain or discomfort were felt (pinprick). The cannula was marked with a piece of paper so that depth of insertion could be measured easily [7].

EMLA cream is a eutectic mixture of lignocaine base 2.5 g dl⁻¹ and prilocaine base 2.5 g dl⁻¹ [12, 13]. The cream was applied under an occlusive dressing (Tegaderm) for 60 min as recommended by the manufacturer and others [13, 14]. Iontophoresis enhances transdermal ionic transport by direct electrical current [4, 15]. In the present experiments, blotting paper was impregnated with 5% lignocaine 0.5 ml with and without adrenaline 1:50 000. Adrenaline was used to provide approximately equal durations of anaesthesia for EMLA cream and iontophoretically applied local anaesthetic [7, 16]. The experiments without adrenaline were performed to study its influence on penetration of iontophoretically applied lignocaine. As the positively charged drugs, lignocaine and adrenaline, travel from anode to cathode, the blotting paper was fixed on the test area under the anode, the cathode being placed on the dorsal side of the forearm. Constant direct current was adapted to the individual tolerance (appearance of stinging or burning sensations) of the volunteers, leading to a current density of 0.1–0.2 mA cm⁻² [4].

Experimental programme

The test areas on each forearm were swabbed with an alcohol solution for degreasing and disinfection. The chosen vein was punctured and 2 ml of

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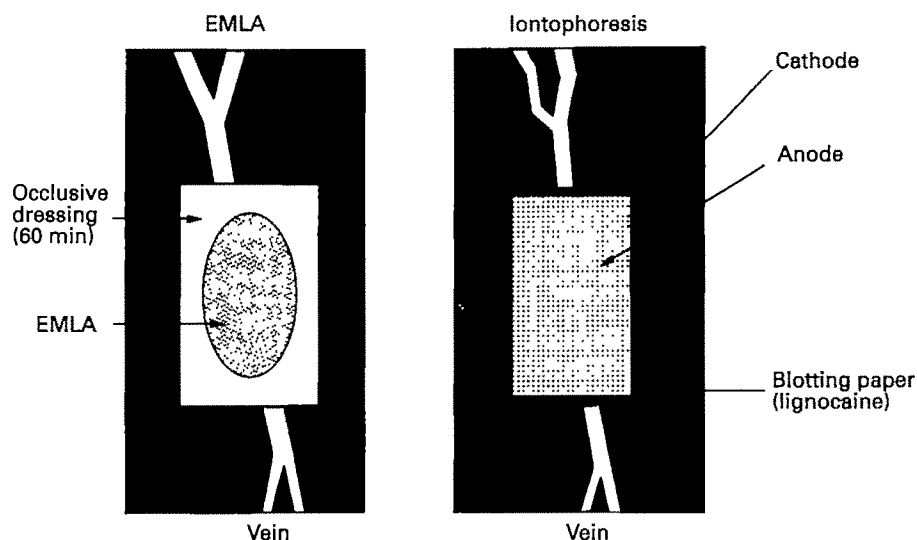


FIG. 1. Schematic presentation of the experimental design. Test areas were of identical size for either application method. EMLA = 2.5% lignocaine + 2.5% prilocaine. Placement of the cathode was on the dorsal surface of the forearm. Anode: 0.1–0.2 mA cm⁻² d.c. for 10 min. Blotting paper was impregnated with 5% lignocaine plus adrenaline 1:50000.

hyperosmolar saline injected as described above, first on the right, then on the left side. Pain on venepuncture and pain intensity on i.v. injection were assessed, the latter being rated on a modified visual analogue scale with the help of an electrically controlled apparatus of our own design [3]. Thus pain could be followed continuously between threshold and tolerance maximum.

After the pain on i.v. injection had subsided, one forearm test area was covered with EMLA cream 2.5 g. On the other forearm, iontophoresis of 5% lignocaine with adrenaline 1:50000 was applied for 10 min [6, 7]. Immediately after removal of EMLA cream and electrodes, sensations during venepuncture and i.v. injection of hyperosmolar saline and depth of cannula insertion were assessed as described above. Depth was investigated every 1–5 min until baseline sensitivity was regained. At that time, a final i.v. injection of hyperosmolar saline was performed to evaluate recovery of sensory innervation of the veins. The experiments with iontophoretically applied plain lignocaine were performed about 2 weeks later under identical conditions.

Data analysis

Pain during injection of hyperosmolar saline (expressed as percent of maximum pain), depth (mm) of painless needle insertion (mean of five measurements at the test sites) and duration of anaesthesia (time (min) from removal of cream or electrodes until full sensitivity to pinprick) were measured for each volunteer or presented as medians and ranges, or both. Data were analysed using Wilcoxon's signed-rank test. Significance was assumed when $P < 0.05$.

RESULTS

In each volunteer, venepuncture was painful before but not after numbing the skin with EMLA cream or

iontophoretically applied local anaesthetic. In contrast, pain on i.v. injection of hyperosmolar saline was significantly reduced only after iontophoresis of lignocaine with adrenaline ($P = 0.04$), but not after EMLA cream ($P = 0.35$). Pain on injection was abolished in five of six volunteers after iontophoresis, whereas pain decreased only slightly in three and increased in three volunteers after EMLA cream (fig. 2). In each subject, anaesthesia as tested by painless insertion of a 27-gauge cannula extended significantly deeper after iontophoresis than after

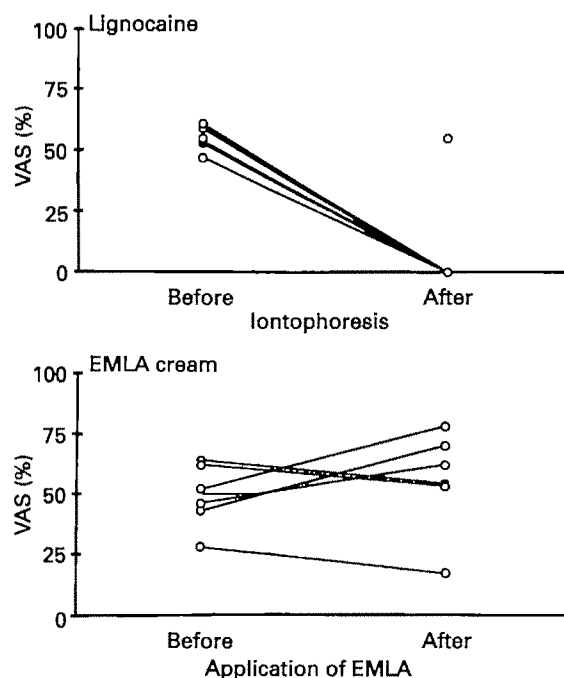


FIG. 2. Pain intensity on i.v. injection of hyperosmolar solution (2 ml of saline 2500 mosmol kg⁻¹) over 10 s, as rated by a modified visual analogue scale (VAS) (range 0% = no pain to 100% = maximal tolerable pain). Pain scores before (left) and after (right) iontophoresis of 5% lignocaine with adrenaline 1:50000 or application of EMLA cream.

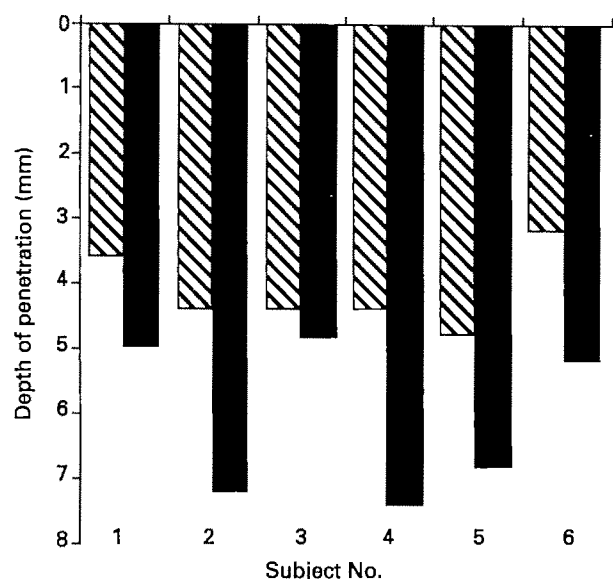


FIG. 3. Depth of painless insertion of a 27-gauge cannula after application of EMLA cream (▨) (mean 4.4 mm; range 3.2–4.8 mm) or iontophoresis of 5% lignocaine with adrenaline 1:50000 (■) (mean 6.0 mm; range 4.8–7.4 mm). Depth of tissue penetration was significantly greater after iontophoresis than after EMLA ($P = 0.03$).

application of EMLA cream (fig. 3). Duration of anaesthesia did not differ between the methods (table I). Depth of painless needle penetration after iontophoretic application of lignocaine both with and without adrenaline did not differ significantly, whereas duration of anaesthesia was significantly prolonged by adrenaline (table I). Iontophoresis of plain lignocaine induced local hyperaemia, but iontophoretically applied lignocaine with adrenaline markedly blanched the anodal skin area.

DISCUSSION

We have found that transdermal local anaesthesia by iontophoresis, but not by EMLA, reduced pain on i.v. injection of hyperosmolar saline, whereas venepuncture was painless with both methods.

The experiments were designed to compare the efficacy of transdermal local anaesthesia by two methods, therefore iontophoresis of local anaesthetics and EMLA cream were applied as recommended in the literature for best effect. Covered with an occlusive dressing for 60 min, EMLA penetrates intact skin. Although application times up to 120 min

were thought necessary in one study [16], EMLA has been shown to be effective after a period as short as 20 min [17]. In accordance with most investigators [9, 14, 18, 19], we exposed the skin for 60 min, as recommended by the manufacturer.

The effect of iontophoretically applied local anaesthetic depends on direct electrical current intensity [7]. In the present experiments, current was increased slowly until discomfort (stinging or burning sensations) was felt, usually at $0.1\text{--}0.2\text{ mA cm}^{-2}$ [4]. Although 2–4% lignocaine is recommended for iontophoresis [7], a 5% solution was used in the present experiments to provide adequate local anaesthetic. Adrenaline was added to lignocaine in one series of experiments to provide a duration of action similar to that of EMLA cream. When testing iontophoretically applied lignocaine with or without adrenaline under otherwise identical conditions, we found that the depth of tissue penetration did not differ. Obviously, the addition of adrenaline influenced the duration of action, whereas depth of tissue penetration depended only on the electrical conditions used during iontophoresis. Other workers have found that the mean depth of penetration of lignocaine with and without noradrenaline into skin, as measured by radioactive tracers, was identical [20].

Peripheral veins are invested with polymodal nociceptors which, in common with cutaneous receptors, respond to hyperosmolar solutions [3]. As only the iontophoretically applied local anaesthetics reduced pain on i.v. injection, iontophoresis obviously forces the molecules deep enough into skin to block vein wall afferents. Depth of painless needle insertion in each volunteer was also greater with iontophoresis than with EMLA cream.

Although five volunteers did not feel any pain after iontophoretic application of lignocaine with adrenaline, pain intensity after i.v. injection of hyperosmolar saline was unchanged in one subject. This volunteer was very sensitive, as demonstrated by the low current (0.1 mA cm^{-2}) necessary to induce stinging or burning sensations. As the effect of iontophoresis correlates with the current applied [4], penetration of lignocaine may have been limited in this volunteer; indeed, he had the least difference in depth of tissue penetration (subject No. 3 in figure 3: 4.4 mm EMLA, 4.8 mm iontophoresis). We believe that the use of a larger current (up to 0.6 mA cm^{-2} as used and tolerated by volunteers in several studies [7, 8, 21]) would have increased depth of anaesthesia and made this particular subject analgesic to i.v. injection of hyperosmolar saline.

TABLE I. Depth and duration of transdermal anaesthesia (median (range)). * Significant difference ($P < 0.05$) compared with EMLA; ns compared with no adrenaline. † Significant difference ($P < 0.05$) compared with no adrenaline; ns compared with EMLA

Iontophoresis: 5% lignocaine

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EFFECT OF METOCLOPRAMIDE ON RENAL VASCULAR RESISTANCE INDEX AND RENAL FUNCTION IN PATIENTS RECEIVING A LOW-DOSE INFUSION OF DOPAMINE

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SUMMARY

Metoclopramide is a dopamine receptor antagonist and, in large doses, has been shown to reverse the increase in renal blood flow, the diuresis and the natriuresis produced by a low-dose infusion of dopamine. We gave a single i.v. dose of metoclopramide 10 mg to 21 patients on an intensive care unit who were haemodynamically stable and receiving dopamine $3 \mu\text{g kg}^{-1} \text{min}^{-1}$. Renal vascular resistance index, measured by duplex Doppler ultrasonography, did not change after metoclopramide. Renal function, measured by creatinine and osmolar clearance, natriuresis, kaliuresis and excretion fractions of sodium and potassium did not change after metoclopramide. We conclude that normal doses of metoclopramide ($< 0.2 \text{ mg kg}^{-1}$) do not antagonize the renal effects of a low-dose infusion of dopamine in critically ill patients (Br. J. Anaesth. 1993; 71: 379–382)

KEY WORDS

Kidney: function, blood flow. Pharmacology: metoclopramide.

Low-dose infusion of dopamine causes renal vasodilatation and is used commonly in critically ill patients in an attempt to preserve renal function [1]. An increase in renal blood flow has been demonstrated by direct blood flow measurements in animals [2], by indicator dilution in man [3], by angiographic and isotope studies in man [4] and inferred non-invasively in man using duplex Doppler [5]. Dopamine also causes an increase in glomerular filtration rate, diuresis and natriuresis [3, 6, 7].

Metoclopramide is used frequently in critically ill patients who are nauseated or have gastric stasis. It acts by antagonizing dopamine centrally and peripherally [8]. Many clinicians are reluctant to prescribe metoclopramide to patients who are receiving dopamine for fear of antagonizing the effect of dopamine on kidney perfusion and tubular function. Renal plasma flow was reduced after high-dose metoclopramide ($1\text{--}2.5 \text{ mg kg}^{-1}$) was given to chemotherapy patients who were not receiving dopamine [9], suggesting that metoclopramide may antagonize endogenous dopamine. This finding was not con-

firmed in healthy volunteers, but the study demonstrated that metoclopramide, given by infusion to a total dose of 40–70 mg, caused a reduction in sodium excretion and attenuation of the dopamine-induced increases in renal plasma flow and glomerular filtration rate [7]. High-dose metoclopramide ($1\text{--}10 \text{ mg kg}^{-1}$) has been shown to inhibit dopamine-induced renal vasodilatation in dogs [10]. Metoclopramide in the usual clinical dose of 10 mg did not affect the renal vascular resistance of volunteers who were not receiving dopamine [11]. There have not been any studies of this dose given to patients receiving dopamine.

Duplex Doppler ultrasonography is a safe, non-invasive and rapid method of studying resistance to blood flow in the renal vasculature and has been used recently in volunteers [11], critically ill patients [5, 12] and patients with renal disease [13]. Several indices have been derived from the duplex Doppler trace of a blood vessel which indicate resistance to flow downstream from the site of recording. From the renal artery and the renal segmental arteries one can measure renovascular resistance. Using a canine model of increased microvascular resistance Norris and Barnes demonstrated a strong correlation between resistance measured by duplex Doppler and by an invasive method [14]. Duplex Doppler has recently been shown to be a useful method of monitoring transplanted kidneys for rejection, and Rasmussen and Pedersen have shown the technique to be precise when the renovascular resistance was measured repeatedly in patients after transplantation [15].

In this study we investigated the effects of metoclopramide in critically ill patients who were receiving dopamine $3 \mu\text{g kg}^{-1} \text{min}^{-1}$. We used duplex Doppler to measure the resistance index (RI) of the renal vasculature. Renal glomerular and tubular function was measured by conventional clearance and excretion fraction measurements.

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PATIENTS AND METHODS

We studied 21 adult patients in the Intensive Therapy Unit. The study was approved by the District Ethics Committee and all patients or next-of-kin gave informed consent. Fifty percent of the patients had undergone major vascular surgery and, among the others, there were equal numbers recovering from major trauma, general surgical and general medical conditions. No patient had been in established renal failure or had undergone any surgery involving the renal tract. All patients had been receiving an i.v. infusion of dopamine $3 \mu\text{g kg}^{-1} \text{min}^{-1}$ for at least 24 h as part of their routine management. They had all demonstrated haemodynamic stability, were in sinus rhythm, had a urinary catheter *in situ* and had not received another inotrope, a diuretic or antiemetic for at least 8 h before the start of the study. No patient was nauseated at the time of the study. The patients were either breathing spontaneously or were undergoing mechanical ventilation of the lungs, but the mode of ventilation was not changed during the study. No patient was receiving continuous positive airway pressure. In those ventilated with positive end-expired airway pressure (PEEP), PEEP was 4 cm H_2O or less and was not changed during the study. Nine of the patients were receiving extradural infusions (0.167% bupivacaine with or without diamorphine) for postoperative analgesia. The patients remained well oxygenated and received i.v. fluid at a constant rate throughout.

Each study lasted for 120 min. The patient's heart rate, arterial pressure and central venous pressure was recorded at 60, 75, 90 and 120 min. Metoclopramide 10 mg i.v. was given at 60 min. RI was measured at 60 min (before metoclopramide) and at 75 and 90 min. Urine was collected for 1 h before metoclopramide and for 1 h after. The bladder was compressed to ensure complete emptying at the end of the collections. A blood sample was taken at 60 min. Renal clearances of creatinine, urea and osmolar load and the excretion rate and excretion fraction of sodium and potassium were calculated for the two 1-h periods before and after administration of metoclopramide.

The patient's kidneys were imaged using a mechanical sector ultra sound scanner, ATL 860C (Advanced Technology Laboratories UK, Letchworth, Herts). The right kidney was scanned because of its anatomical site and ease of imaging. If the right kidney could not be imaged well, the left kidney was used. The Doppler sample volume was used to scan the upper or lower pole of the kidney until the strongest consistent signal was obtained from an interlobar artery. The same point was chosen for subsequent recordings in that patient. All three sets of recordings in each patient were carried out by the same observer. The Doppler signal was analysed by a dedicated Doppler spectrum analyser, Dopstation (Scimed, Bristol, U.K.), and the frequency shift spectra recorded and stored on computer. Each set of recordings consisted of at least 25 cardiac cycles, although normally more than 50 cardiac cycles were recorded and analysed. All readings were taken in

TABLE I. Mean (SD) renal resistance index (RI) in 16 patients, measured by duplex Doppler before and after metoclopramide (metocl.) 10 mg. No significant differences

	Before metocl.	After metocl.	
	60 min	75 min	90 min
RI	0.671 (0.078)	0.674 (0.074)	0.68 (0.07)

expiration. The Doppler recordings were subsequently analysed, and RI calculated, by one observer who was blinded to the timing of the recording relative to the injection of metoclopramide.

RI is the ratio of the difference between the Doppler peak systolic frequency shift and the end-diastolic frequency shift divided by the peak systolic frequency shift. The smaller the RI, the less the impedance to flow downstream from the site of recording:

$$\text{RI} = \frac{\text{peak systolic frequency shift} - \text{diastolic frequency shift}}{\text{peak systolic frequency shift}}$$

RI results and haemodynamic measurements were compared using analysis of variance for repeated measures. Results of the renal function tests for the 1-h periods before and after administration of metoclopramide were compared using the Wilcoxon signed-rank test.

RESULTS

Doppler recordings were obtained from 16 patients and full biochemical results from 17 patients.

Heart rate, central venous pressure and systolic, mean and diastolic arterial pressures remained unchanged throughout the study.

There was no difference in mean RI before injection of metoclopramide at 60 min (0.671 (SD 0.078)) and after metoclopramide at 75 min (0.674 (0.074)) and 90 min (0.68 (0.07)) (table I).

Neither urine volume nor any renal function tests differed when the 1-h periods before and after administration of metoclopramide were compared (table II).

DISCUSSION

We found that metoclopramide did not affect RI of the renal vasculature in patients who were receiving a low-dose infusion of dopamine. Measures of renal function were also unchanged by metoclopramide. In this study we did not attempt to demonstrate any change in renal vascular RI or renal function produced by dopamine because the patients were haemodynamically unstable at the time the dopamine infusion was started, which was at least 24 h earlier. However, many investigators have shown that low-dose dopamine causes renal vasodilatation [2-5] and increases in glomerular filtration rate, diuresis and natriuresis [3, 6, 7]. The differences between our results and others which have shown that metoclopramide reduced the renal effects of dopamine in

TABLE II. Renal function tests (normal range and median (range)) in 17 patients for 1-h periods before and after metoclopramide 10 mg. No significant differences

Renal function test	Normal range	Before	After
Urine volume (ml h ⁻¹)	> 30	60 (22–200)	63 (23–193)
Creatinine clearance (ml s ⁻¹ /1.73 m ² BSA)	1.24–2.08	1.43 (0.52–3.51)	1.49 (0.7–3.0)
Osmolar clearance (ml s ⁻¹)	0.033–0.066	0.028 (0.012–0.094)	0.036 (0.014–0.095)
Sodium excretion (mmol h ⁻¹)	2.08–8.33	3.02 (0.4–17.0)	3.42 (0.34–32.4)
Excretion fraction sodium (%)	0.4–1.25	0.4 (0.056–2.76)	0.38 (0.061–2.63)
Potassium excretion (mmol h ⁻¹)	1.04–4.17	3.06 (1.58–17.0)	3.13 (1.82–9.1)
Excretion fraction potassium (%)	4–19	17.13 (7.13–42.63)	15.36 (8.41–37.41)

animals [10, 16] and in man [7] could be explained by the fact that we gave metoclopramide by the usual method of administration (slow i.v. injection) and in the usual clinical dose (10 mg). Previous investigators gave metoclopramide in very large dosage (between 1 and 10 mg kg⁻¹) or by infusion. Similarly, in patients who were not receiving dopamine, renal plasma flow decreased after large doses of metoclopramide [9], but renal RI in volunteers did not change after metoclopramide 10 mg [11].

These differences between our findings and those of previous investigators could be explained by a dose-dependent effect of metoclopramide on the two dopamine receptor subtypes. Dopamine has several different actions in the kidney, including vasodilatation [2–5] and inhibition of the tubular Na/K-ATPase pump [17], which are mediated by DA-1 receptors. Stimulation of DA-2 receptors reduces the release of noradrenaline from sympathetic nerve endings, causing central nervous system and endocrinological effects, including inhibition of release of aldosterone [18, 19]. Metoclopramide has been classified as a DA-2 receptor antagonist [19], or as a D-2 antagonist using Kebabian's classification of dopamine receptors [18], but this may be true only in small doses [20]. It is possible that high-dose metoclopramide also antagonizes the DA-1 receptor, which would explain the results reported previously. Dopamine also acts at adrenergic receptors, with beta effects predominating in the dose range 5–10 µg kg⁻¹ min⁻¹ and alpha effects with doses greater than 10 µg kg⁻¹ min⁻¹. There is a large inter-patient variability and it is probable that dopaminergic and adrenergic receptors are active to some extent throughout the dose range [19]. However, although dopamine has inotropic effects in some patients at infusion rates as small as 1 µg kg⁻¹ min⁻¹, the increase in renal blood flow and natriuresis which occurs is proportionately much greater than the increase in cardiac output [3].

The Doppler frequency shift has been used in other studies [5] to investigate changes in renal blood flow. This measurement requires knowledge of the anatomy of the artery and the angle between the ultrasound beam and the artery being studied. In this study, we could not be certain of imaging the same artery at the same angle for each set of

recordings, so we did not measure frequency shift. We were thus not attempting to measure either blood flow velocity or renal blood flow volume, but only changes in resistance to flow. Resistance index is a ratio that is independent of the angle of interrogation of the vessel studied. Pulsatility index is another index of resistance derived from the duplex Doppler trace which has been used to measure renovascular resistance [15, 21], but it is more dependent than resistance index on heart rate, and for this reason we chose not to use it in this study.

Plasma clearances were measured for 1-h periods, instead of 12 or 24 h. All patients in this study had urinary catheters *in situ*, thus minimizing the possibility of incomplete bladder emptying, which may produce inaccuracies in short urinary clearance studies. Provided that the urine output is reasonably constant, inaccuracies in this method are minimal. Only one blood sample was used, as opposed to one during each hour, as it was considered that any differences in serum concentrations occurring over 1 h were likely to be so small as to be within the limits of laboratory error. One-hour and 2-h creatinine clearance studies have been shown to be reproducible and comparable to 24-h studies in the healthy population and in the critically ill, provided that such studies are carefully controlled [22–24].

There were wide ranges in the results for all the tests of tubular function. The median results for some tests were just outside the normal range for the healthy population. We believe that this reflects our heterogeneous patient group, different degrees of physiological stress and effects of dopamine.

Nine of the patients had functioning extradurals for the duration of the study. The extradural infusions were not changed and produced no haemodynamic changes during the study. Previous studies have demonstrated that initiation of an extradural block affecting the sympathetic outflow to the kidney and adrenal medulla (T6–L2) may reduce glomerular filtration rate and renal plasma flow by reducing arterial pressure [25], although evidence for a direct effect on the renal vasculature is conflicting. There is no evidence that a continuous extradural infusion causes fluctuations in renal blood flow if the patient is haemodynamically stable. It is not known if the

effect of dopamine is altered if there is a sympathetic block causing renal vasodilatation.

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COMPARISON OF CNS EFFECTS OF PROPOFOL AND THIOPENTONE IN CATS

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SUMMARY

Using cats with chronically implanted brain electrodes, we have compared the effects of propofol on CNS electrical activities with those of thiopentone. Ten cats were allocated to receive either propofol ($n = 5$) or thiopentone ($n = 5$). Cats were anaesthetized initially with 4% halothane in oxygen. The trachea was intubated and the lungs ventilated mechanically. A femoral artery and a vein in a forepaw were cannulated for arterial pressure monitoring and fluid infusion. After the inspired concentration of halothane was maintained at 0.5%, EEG in the cortex, the amygdala and the hippocampus, somatosensory evoked potential (SEP) and reticular multi-unit activity (R-MUA) were recorded. Incremental doses of propofol or thiopentone were administered i.v. at 5-min intervals during 0.5% halothane anaesthesia. The cumulative doses of propofol and thiopentone were 2, 5, 10 and 20 mg kg⁻¹, and 4, 10, 20 and 40 mg kg⁻¹, respectively. Arterial pressure was maintained in excess of 100 mm Hg systolic by infusion of phenylephrine. All cats in the propofol group survived, but two in the thiopentone group died after the administration of thiopentone 40 mg kg⁻¹. Changes observed in CNS activity were dose-related in all cases. The EEG changed with the increments of doses of propofol or thiopentone, from fast, small amplitude to complexes of fast and slow, large amplitude activities, burst suppression and flat EEG. SEP latency was prolonged by both agents: the peak latency of N1 changed from 15 (SD 2) ms to 20 (5) ms with propofol 20 mg kg⁻¹, and from 14 (1) ms to 27 (2) ms with thiopentone 40 mg kg⁻¹. SEP amplitudes were depressed by both agents: the amplitude of N1 was depressed by 70 (29)% with propofol 20 mg kg⁻¹ and by 60 (33)% with thiopentone 40 mg kg⁻¹. The R-MUA also was depressed by both agents: 85 (4)% with propofol 20 mg kg⁻¹ and 85 (8)% with thiopentone 40 mg kg⁻¹. The R-MUA was depressed to 50% of control by propofol 3.2 (1.6) mg kg⁻¹ or thiopentone 6.7 (5.0) mg kg⁻¹. These depressive actions on the EEG, SEP and R-MUA induced by propofol were similar to those induced by twice the equivalent doses of thiopentone on an mg kg⁻¹ basis. These results indicate that propofol has the same simple depressant effects as thiopentone on CNS electrical activity. (Br. J. Anaesth. 1993; 71: 383–387)

KEY WORDS

Anaesthetics, intravenous: propofol, thiopentone. Brain: EEG, multi-unit activity, somatosensory evoked potential.

Propofol is an i.v. anaesthetic which is used for both anaesthesia and sedation in ICU patients [1, 2]. Our laboratory has shown that anaesthetics exert not only depressant, but also some stimulant actions on the CNS [3, 4]. In the present study, we have attempted to determine if propofol has CNS stimulating actions. The CNS effects were compared with those of thiopentone, which represents a pure CNS depressant.

METHODS

After approval by the Institutional Committee on Animal Research, we studied 10 cats (both sexes, weights 2.5–3.5 kg), anaesthetized with pentobarbitone 40 mg kg⁻¹ i.p. for placement of electrodes at least 1 week before the study. Stainless steel screws, 2.0 mm in diameter, were inserted in the frontal bone as reference electrodes and over the primary sensory cortex and temporal cortex to record the somatosensory evoked potential (SEP) and the cortical EEG. Parallel stainless steel wire electrodes, 0.2 mm in diameter, insulated with epoxylite resin except the cut-end tips, with a vertical separation of 0.5–1.0 mm at the tips, were inserted bilaterally in the midbrain reticular formation (A2; L3; H-2, according to the atlas of Snider and Niemer [5]) to record the reticular multi-unit activity (R-MUA). The same wire electrodes were inserted in the dorsal hippocampus (A2; L8; H9) and in the medial amygdala (A12; L9; H6). The electrodes were connected to a socket which was fixed to the skull with dental cement.

The cats were anaesthetized in a 50-litre anaesthesia box, with 4% halothane in oxygen. Tracheal intubation was performed with the aid of alcuronium, initial dose 1 mg i.v., supplemented as required during the study. The lungs were ventilated mechanically using a non-rebreathing method (Acoma Animal Respirator, AR-300) and anaesthesia was maintained with 1% halothane in oxygen during the preparation. A cannula was inserted into the

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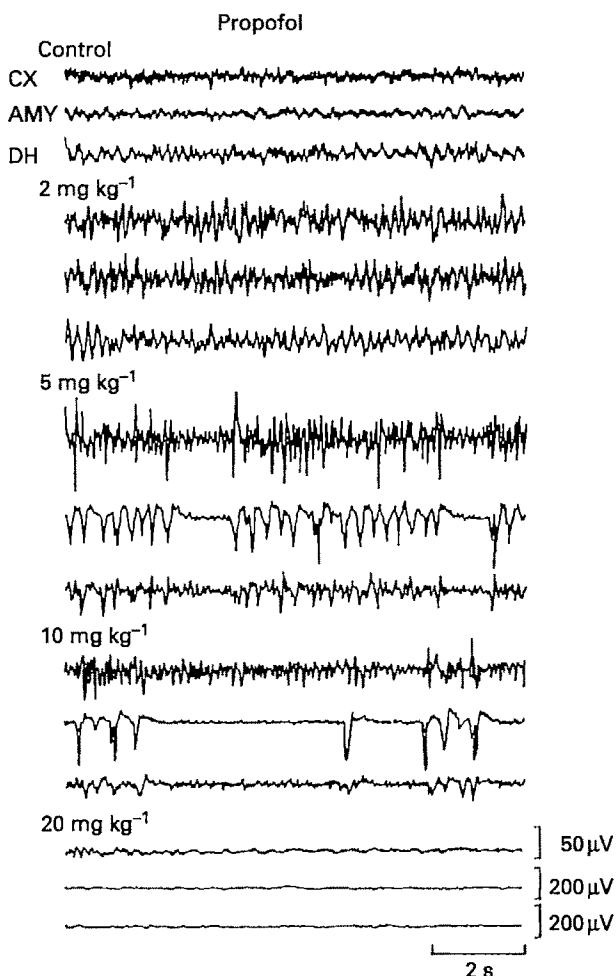


FIG. 1. Effects of propofol on EEG in the cat. CX = Cortex; AMY = medial amygdala; DH = dorsal hippocampus. Basal anaesthesia was maintained with 0.5% halothane in oxygen. Control EEG were small amplitude fast activity. Amplitudes increased and background activity slowed with propofol 2 mg kg^{-1} . Burst suppression patterns appeared with propofol 5 mg kg^{-1} and the period of suppression increased with propofol 10 mg kg^{-1} . Almost flat EEG occurred with propofol 20 mg kg^{-1} .

femoral artery to permit monitoring of arterial pressure and sampling blood for gas analysis and measuring the concentrations of propofol; another was placed in a forepaw vein for administrations of fluids and drugs. For peripheral nerve stimulation, two needles were placed, 1 cm apart, subcutaneously near the radial nerve in the forepaw contralateral to the screw placed over the primary sensory area. The inspired concentration of halothane was decreased to 0.5% in oxygen and was maintained at this value during the study. End-tidal carbon dioxide partial pressure was measured using an infra-red carbon dioxide monitor (Nihonkoden, CO_2 monitor OIR-7101) and was maintained in the range 4.0–4.6 kPa by adjusting tidal volume. Blood-gas analysis was performed before administration of propofol or thiopentone, and pH 7.40–7.44 maintained using sodium bicarbonate i.v. A rectal thermistor (Termo-Finer, TN-DN) was inserted and temperature maintained at 37–39 °C with a warm water mattress and heating lamp. Arterial pressure was maintained in excess of 100 mm Hg systolic by administration of phenylephrine.

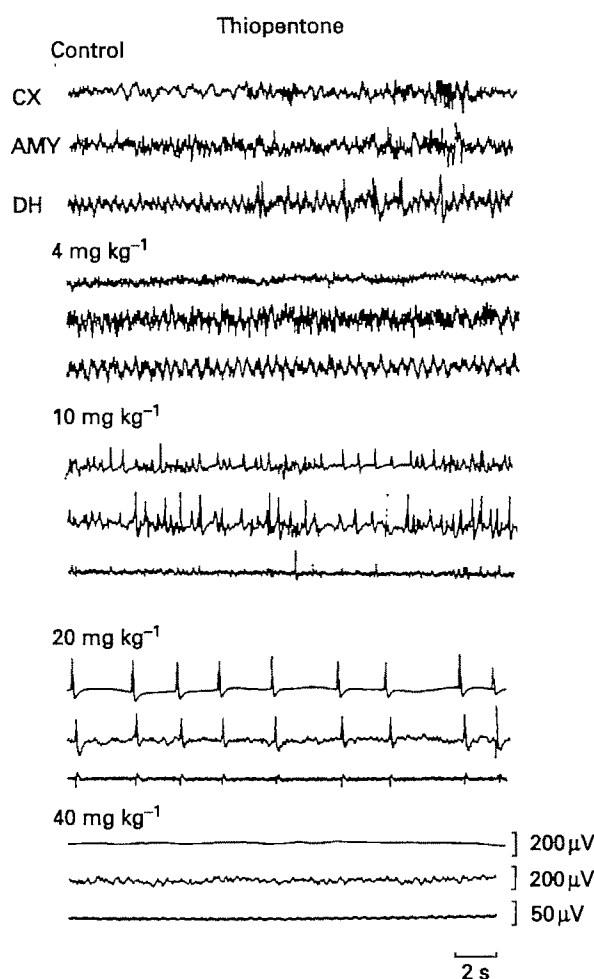


FIG. 2. Effects of thiopentone on EEG in the cat. CX = Cortex; AMY = medial amygdala; DH = dorsal hippocampus. Basal anaesthesia was maintained with 0.5% halothane in oxygen. Spindles were observed in the control cortical EEG. Cortical EEG demonstrated small amplitude fast activity with thiopentone 4 mg kg^{-1} , while hippocampal EEG showed rhythmic theta activity. Burst suppression patterns with fast waves appeared with thiopentone 10 mg kg^{-1} and synchronized sharp waves occurred and the periods of suppression increased with 20 mg kg^{-1} . Almost flat EEG were observed with thiopentone 40 mg kg^{-1} .

EEG were recorded on an eight-channel polygraph (Sanei polygraph Recti-Horiz 8K), and R-MUA and arterial pressure on an oscillograph (Sanei Rectigraph 8S). SEP were evoked by supramaximal (approximately 10 V) square-wave pulses of 0.5 ms delivered manually. Ten responses were summated, using a computerized signal processor (Sanei 7S06A), and the resulting traces were plotted on an X-Y plotter (Watanabe WX442). Latencies of P1 and N1 were measured from the application of stimuli to the peaks. Amplitudes of components were measured as the vertical distances from the pre-stimulus level to the peak of components and expressed as percent of control. The firing of a population of reticular neurones was measured using a multi-unit activity technique described previously [6]. Briefly, the technique was as follows. The wide-band signal obtained from the preamplifier of the polygraph was passed to a high frequency band-pass filter, the peak frequency response of which was 1300 Hz, with 30 dB attenuation at 600 and 2500 Hz. As the conventional ink-writing oscillograph could

not follow such high frequency activities, the output was rectified and smoothed with an electronic circuit, with a smoothing time constant of 50 ms, and expressed as an oscillation in d.c. value: the greater the d.c. value the greater the firing of a population of units. This signal was recorded on a slow-moving oscillograph (Sanei Rectigraph 8S) at a paper speed of 5 mm min⁻¹. The noise level of the recording system was estimated by the difference between the d.c. levels obtained by inserting either a 10 k Ω resistor or a short across the input in place of the animal. The signal was measured as the distance from the lower limit of rectified trace to the 10-k Ω resistor line. The signal to noise ratio exceeded 10 in all cases. By this recording technique, neuronal discharges were picked up from an area of approximately 1 mm radius around the tip of the electrode [7]. As the multi-unit activity recorded is dependent, not only on the amount of bioelectric activity, but also on the impedance of electrodes, calibration of MUA was not practical, and changes in the lower limit of spontaneous activity of the MUA trace with the administration of propofol or thiopentone were measured and expressed as percent of activity in the control period. Arterial blood concentrations of propofol were measured using high pressure liquid chromatography (HPLC, Yanaco L-4000W) with fluorescence detection (Shimadzu RF-530) by a modification of the method of Plummer [8].

Cats were allocated to two groups: propofol ($n = 5$) or thiopentone ($n = 5$). The doses of propofol and thiopentone for induction of anaesthesia clinically are 2–2.5 and 4–4.5 mg kg⁻¹, respectively, therefore we selected the ratio of 1 (propofol): 2 (thiopentone) on an mg kg⁻¹ basis for comparisons. The cumulative doses studied were 2, 3, 5 and 10 mg kg⁻¹ in the propofol group and 4, 6, 10 and 20 mg kg⁻¹ in the thiopentone group, administered cumulatively at 5-min intervals. One hour after the inspired concentration of halothane was changed to 0.5%, control values were obtained. Measurements and arterial blood samples were obtained 2 min after administration of each dose.

Values were expressed as mean (SD). The dose by which R-MUA was depressed to 50% of control (ED₅₀), was calculated by the interpolation method in each cat. Effects of the agents were analysed statistically using repeated measures analysis of variance and paired Student's t tests with Bonferroni's correction, and were compared with control values. Comparisons of effects between propofol and thiopentone were analysed using unpaired Student's t tests, and the ratio of the compared doses between agents was 1 (propofol): 2 (thiopentone). $P < 0.05$ was considered significant.

RESULTS

The systolic arterial pressure was maintained in excess of 100 mm Hg in all cats which received propofol. However, two cats of five which received thiopentone died after administration of thiopentone 40 mg kg⁻¹, during phenylephrine infusion.

The arterial concentrations of propofol 2 min after cumulative administration were 2.01 (0.55), 4.28

(0.86), 8.58 (2.37) and 21.61 (7.04) $\mu\text{g ml}^{-1}$ with propofol 2, 5, 10 and 20 mg kg⁻¹, respectively. The correlation between blood concentration and the dose was significant ($P < 0.001$; $r = 0.908$; $n = 20$).

All CNS activities responded to each agent with dose-related changes. Cortical EEG changed from fast activities to a mixture of fast and slow large amplitude activities, burst suppression and finally flat recording (figs 1, 2); the burst suppression patterns were induced with propofol 5 mg kg⁻¹ or thiopentone 10 mg kg⁻¹. EEG in the deep structures showed changes similar to those in the cortical EEG (table I, figs 3, 4). R-MUA at maximum doses of propofol and thiopentone was 15 (4)% and 15 (8)% of control. ED₅₀ for propofol and thiopentone were 3.2 (1.6) mg kg⁻¹ and 6.7 (5.0) mg kg⁻¹, respectively.

TABLE I. Depression of midbrain reticular cell firing (R-MUA) by propofol or thiopentone during halothane anaesthesia (mean (SD)). Control values = 100%. $n = 5$, except for thiopentone 40 mg kg⁻¹, for which $n = 3$. * $P < 0.05$ compared with control

Propofol (mg kg ⁻¹)	0	2	5	10	20
R-MUA (%)	100	59 (12)*	36 (12)*	23 (5)*	15 (4)*
Thiopentone (mg kg ⁻¹)	0	4	10	20	40
R-MUA (%)	100	55 (14)*	37 (18)*	25 (9)*	15 (8)*

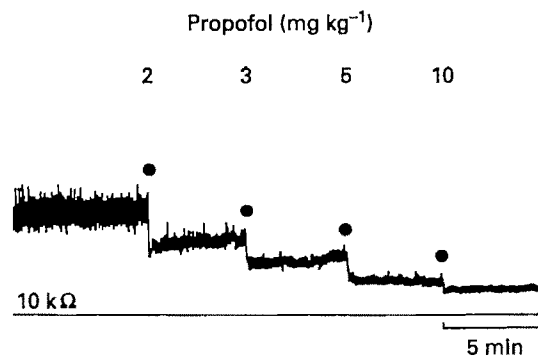


FIG. 3. Effects of propofol on midbrain reticular multi-unit activity (R-MUA) in the cat. Propofol was administered at ●; cumulative doses were 2, 5, 10 and 20 mg kg⁻¹. R-MUA was measured as height from the line assigned 10 k Ω . Propofol depressed R-MUA in a dose-dependent manner.

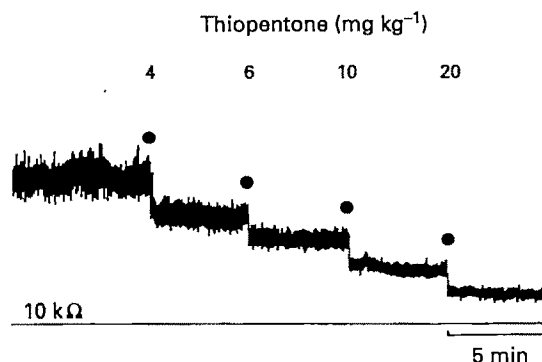


FIG. 4. Effects of thiopentone on R-MUA in the cat. Thiopentone was administered at ●; cumulative doses were 4, 10, 20 and 40 mg kg⁻¹. Thiopentone depressed the R-MUA in a dose-dependent manner.

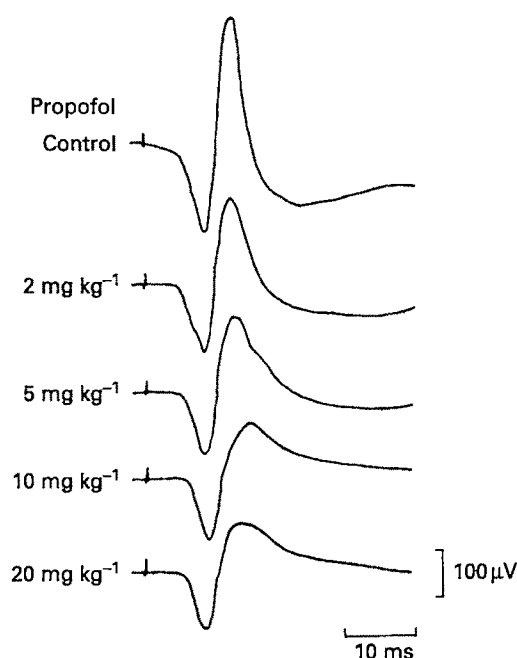


FIG. 5. Effects of propofol on somatosensory evoked potential (SEP) in the cat. The downward deflection indicates positive. The SEP consisted of two waves: the initial downward deflection (P1) and succeeding upward deflection (N1). Propofol suppressed the amplitude of SEP in a dose-dependent manner.

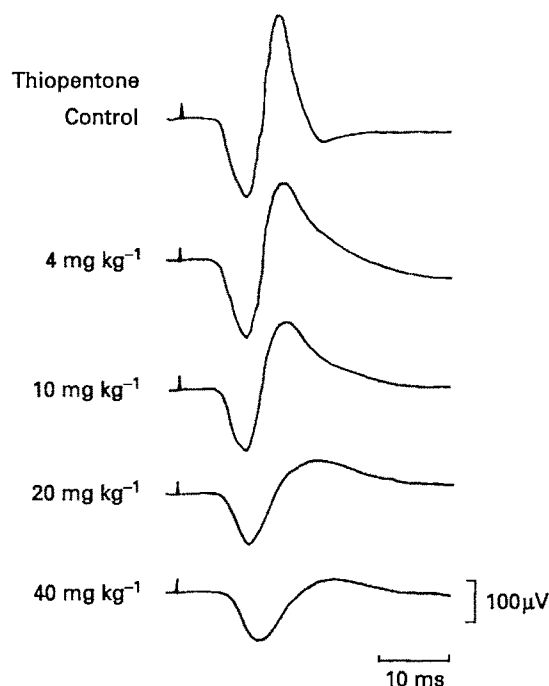


FIG. 6. Effects of thiopentone on SEP in the cat. Thiopentone suppressed the amplitude of SEP in a dose-dependent manner; peak latencies prolonged with increasing doses of thiopentone.

TABLE II. Effects of propofol and thiopentone on the somatosensory evoked potential (P1) (mean (SD)). $n = 5$, except for thiopentone 40 mg kg^{-1} for which $n = 3$. * $P < 0.05$; ** $P < 0.01$ compared with control

Propofol (mg kg^{-1})	0	2	5	10	20
Latency (ms)	10.0 (1.3)	10.2 (1.4)	10.4 (1.2)	10.5 (1.1)	10.5 (1.3)
Amplitude (%)	100	90 (11)	84 (10)*	85 (8)*	75 (14)**
Thiopentone (mg kg^{-1})	0	4	10	20	40
Latency (ms)	9.4 (0.4)	9.5 (0.6)	9.8 (0.5)	10.0 (0.7)*	12.2 (1.2)*
Amplitude (%)	100	106 (18)	81 (16)	78 (16)*	78 (25)

TABLE III. Effects of propofol and thiopentone on the somatosensory evoked potential (N1) (mean (SD)). $n = 5$, except for thiopentone 40 mg kg^{-1} for which $n = 3$. * $P < 0.05$; ** $P < 0.01$ compared with control

Propofol (mg kg^{-1})	0	2	5	10	20
Latency (ms)	15.0 (2.3)	16.5 (3.1)	19.0 (5.1)*	20.1 (4.7)**	20.3 (5.4)**
Amplitude (%)	100	71 (13)*	54 (18)*	47 (20)**	30 (29)**
Thiopentone (mg kg^{-1})	0	4	10	20	40
Latency (ms)	13.7 (0.6)	14.3 (1.2)	18.4 (3.1)*	22.8 (2.1)*	26.9 (2.3)**
Amplitude (%)	100	119 (14)	70 (23)**	56 (14)**	40 (23)*

Effects of propofol on SEP were also similar to those of thiopentone: latencies were prolonged and amplitude decreased (figs 5, 6, tables II and III). There was no significant difference between effects on the SEP produced by propofol and by twice the equivalent dose of thiopentone.

DISCUSSION

We have found that the effects of propofol on the EEG, midbrain reticular cell firing and SEP were similar to those of thiopentone. These results suggest that propofol is also a simple CNS depressant.

The basal anaesthesia in the present study comprised halothane in oxygen. We have reported previously that halothane is a simple CNS depressant

[4, 5, 9], which suggests that the depressant effects of propofol and thiopentone were additive to that of halothane in the present study. However, the inspired concentration of halothane was small, 0.5%, and the influence of halothane should be small. We administered propofol or thiopentone cumulatively at 5-min intervals and measurements were obtained after 2 min. Propofol has been reported to be metabolized more rapidly and to have less cumulative effects after repeated bolus doses than thiopentone or thiamylal [10–12]. Therefore, a comparison of the effects of the two agents on the basis of identical time schedules might not be appropriate. However, there is a report which indicates that an index of cumulative effects of propofol differs between species [13]: the "utilization rate", calculated by dividing

the total dose administered by the duration of anaesthesia with repeated or continuous administration of propofol, was reported to be small ($0.19 \text{ mg kg}^{-1} \text{ min}^{-1}$) in cats, large ($2.22 \text{ mg kg}^{-1} \text{ min}^{-1}$) in mice and intermediate ($0.61 \text{ mg kg}^{-1} \text{ min}^{-1}$) in rats. The small value in cats suggests that metabolism of propofol is less in cats than in other animal species. It was speculated that cats were deficient in their ability to conjugate phenols. Although the mechanism underlying the cumulative effects of propofol in cats is uncertain, the effects of propofol in cats may not diminish as rapidly as in other animal species. The depression of R-MUA by propofol in the present study did not lessen during the 5-min period after each administration of propofol (fig. 5), which was similar to the effect with thiopentone. We observed no difference between propofol and thiopentone in the recovery of R-MUA depression induced by bolus injections [unpublished observation].

EEG changes with doses of propofol smaller than those used in the present study had been reported by several investigators in rats [13] and in humans [14–16] and are in accordance with findings of the present study. The blood concentrations of propofol associated with burst suppression in the EEG were reported to be greater than $8 \mu\text{g ml}^{-1}$ [17], $5.4 \mu\text{g ml}^{-1}$ during fentanyl–nitrous oxide–propofol anaesthesia and $6.3 \mu\text{g ml}^{-1}$ during fentanyl–propofol anaesthesia [18] in humans—values greater than ours ($4.28 \mu\text{g ml}^{-1}$). This small effective blood concentration of propofol in our study must reflect an additive action of the halothane used to provide background anaesthesia in our study. Glen [13] reported dissociation between effects on the EEG and hypnotic action induced by propofol and thiopentone: although burst suppression was produced by propofol in a dose close to the median hypnotic dose (HD_{50}), thiopentone induced burst suppression in a dose in excess of $2 \times \text{HD}_{50}$ in rats. Glen reported an anaesthetic dose ratio of propofol:thiopentone of 1:1.8, which indicated that the dose ratio inducing burst suppression was 1:3.6. However, the ratio of these doses in our study was 1:2, which is similar to the ratio of clinical potency. Although we did not study the hypnotic effects of propofol or thiopentone and there might be species differences, we suspect that there is no dissociation between propofol and thiopentone in the ratio of their potencies of hypnotic actions to EEG actions.

The effects of propofol on the SEP were similar to those found by Maurette and colleagues [19], who reported prolongation of latencies and depression of amplitudes of SEP by propofol in humans.

The ratio of potency of propofol to that of thiopentone on the CNS in the present study (2.1:1) was close to the anaesthetic ratios reported by Glen (1.8:1) [13] and by Grounds, Moore and Morgan (1.6:1) [20] and that of 2:1 used in the induction of anaesthesia in clinical practice [1, 2]. We conclude

that propofol has simple depressant actions on the activities in the CNS similar to those of thiopentone, and that potency of depression by propofol is twice that of thiopentone on a dose per body weight basis.

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EFFECTS OF SEDATION PRODUCED BY THIOPENTONE ON RESPONSES TO NASAL OCCLUSION IN FEMALE ADULTS

T. NISHINO AND T. KOCHI

SUMMARY

To test the hypothesis that full wakefulness is an important factor in the control of the route of breathing in adult humans, we have studied the responses to nasal occlusion before and during sedation with thiopentone in 14 female subjects. A tightly fitting partitioned face mask separated the nasal and oral breathing routes. Nasal and oral breathing were identified from changes in carbon dioxide concentration and airway pressure in the different compartments of the face mask. Arterial oxygen saturation (Sp_{O_2}) was monitored simultaneously. Eleven of 14 subjects breathed only through the nose (nasal breathers) both before and during sedation. In these subjects, the time required to initiate oral breathing in response to nasal occlusion during sedation was significantly longer than that before sedation (mean 37.7 (SD 15.5) s vs 3.2 (1.3) s ($P < 0.01$)). Also, there was a significant difference ($P < 0.01$) in the smallest values of Sp_{O_2} attained during nasal occlusion before (98.0 (0.8) %) and after (89.3 (4.3) %) sedation. In adult humans the ability to maintain adequate ventilation by switching from the nasal to the oral route in response to nasal occlusion is greatly impaired during sedation, probably because of the impairment of conscious influence on the control of the palatal muscles. (Br. J. Anaesth. 1993; 71: 388–392)

KEY WORDS

Airway: obstruction. Ventilation: obstruction.

Ability to change from the nasal route of airflow to the oral route during nasal obstruction is crucial for maintenance of adequate ventilation. Although the physiological mechanisms underlying the change from nasal to oral passage are not entirely clear, the change of breathing route would depend, not only on reflexes, but also on voluntary control of the palatal muscles. It is generally believed that airway obstruction during sedation and anaesthesia occurs at the pharynx and larynx, because of depression of the upper airway dilating muscles [1]. However, it is possible also that, even in the presence of a patent upper airway, alterations in consciousness caused by sedation or anaesthesia may diminish or abolish the ability to change the breathing route and lead to

airway obstruction during nasal occlusion. The recent study by Wood and Harding [2] showed, in neonatal and mature sheep, that sedating doses of pentobarbitone and diazepam depressed the effectiveness of oral breathing when the nose was blocked. It is not clear if nasal occlusion leads to prompt alterations of breathing route from the nose to the mouth in sedated humans. Consequently, we have examined the responses of adult humans to acute nasal obstruction during wakefulness and after sedation with thiopentone.

PATIENTS AND METHODS

We studied 14 female patients aged 25–48 yr (mean 37.8 yr) undergoing elective surgery (hysterectomy, nine patients; mastectomy, five patients) under general anaesthesia. All subjects were devoid of respiratory, cardiovascular, laryngeal or neuromuscular disorders. None had a history or clinical evidence of partial nasal occlusion, nasal pathology or nasal trauma. The average heights and weights were 157.0 (SD 2.8) cm (range 154–164 cm) and 54.4 (2.8) kg (range 49–58 kg), respectively. The study was approved by the Ethics Committee of our institution, and each subject gave informed consent. The patients received atropine 0.5 mg i.m. 1 h before the study, which was carried out in a quiet, warm operating room.

Each patient rested in the supine position for 10 min with the head resting on a ring support in a comfortably neutral position and an air-tight custom-made, partitioned face mask (Senkousha, Japan) was fitted. A hard rubber septum inside the mask separated nasal and oral chambers, each with its own opening port (i.d. 9 mm, length 40 mm) having a side-arm outlet (i.d. 3.5 mm). The rubber septum was positioned under the upper lip and on the outer gum of the patient so that the mouth and lips were kept slightly apart. Each mask chamber was tested separately for air leaks by pressurizing to ± 10 cm H_2O and ensuring that the pressure was constant for 10 s. Dead-space for nasal and mouth chambers were 90 and 120 ml, respectively. Nasal

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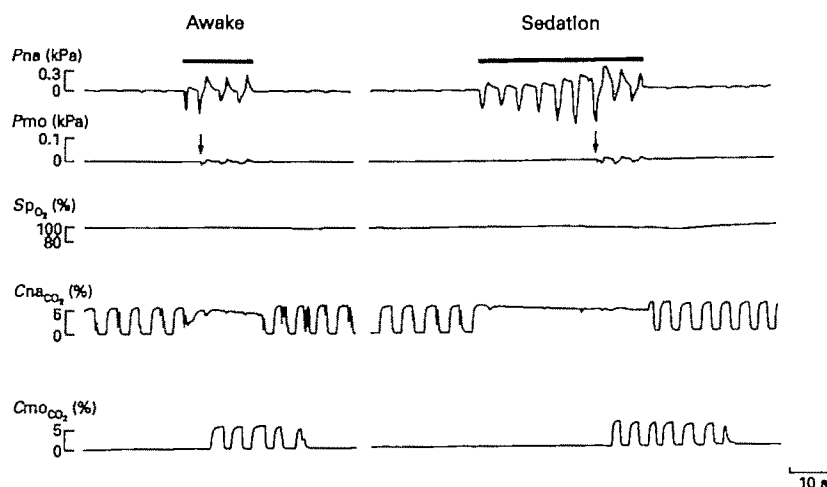


FIG. 1. Experimental records of respiratory responses to nasal occlusion during the awake state and sedation. P_{na} = Nasal pressure; P_{mo} = mouth pressure; Sp_{O_2} = arterial oxygen saturation; C_{naCO_2} = carbon dioxide concentration at the nose; C_{moCO_2} = carbon dioxide concentration at the mouth. Horizontal bars indicate periods of nasal occlusion; arrow indicates start of oral breathing.

pressure (P_{na}) and mouth pressure (P_{mo}) were measured using pressure transducers (Statham P231D) at the opening ports. Carbon dioxide concentrations in the nasal chamber (C_{naCO_2}) and the mouth chamber (C_{moCO_2}) were monitored with Datex infra-red carbon dioxide analysers. In this way, nasal breathing was reflected in the values of P_{na} and C_{naCO_2} , whereas oral breathing was reflected in P_{mo} and C_{moCO_2} . Arterial oxygen saturation (Sp_{O_2}) was monitored continuously using a pulse oximeter (Nihon Kohden, Japan). All variables were recorded on a Nihon Kohden 8 channel recorder (RJG-4128).

The patient was asked to close the eyes and breathe as normally as possible for 3–5 min without instruction as to the breathing route, while listening to music through headphones. During this period, the patients who breathed only through the nose were designated “nasal breathers”. The patients who breathed through both the nose and mouth were designated “oronasal breathers”.

To determine the ability to change the breathing route in response to nasal occlusion, in nasal breathers the opening of the nasal chamber was occluded suddenly during one expiration in such a manner that the subject was unable to detect the onset of application of occlusion. Nasal occlusion was continued until three consecutive oral breaths ensued, after which time the occlusion was released. Three nasal occlusions were performed in each patient and the response time required to start oral breathing after nasal occlusion was measured in each trial period. Each trial period was separated by 1 min; three results were averaged for each patient. Immediately thereafter, each patient received a sedative dose of thiopentone 1.5 mg kg^{-1} i.v. Two minutes after administration of thiopentone, the level of consciousness in each patient was determined by physical stimulation and verbal commands; the anterior chest wall of the patient was tapped gently and the patient asked to open the eyes and squeeze the hands. When the patient was capable of rational responses, nasal occlusion was performed in those

patients who continued to breathe only through the nose after administration of thiopentone. The response time required to start oral breathing after nasal occlusion was measured and this value was compared with those obtained in awake state.

Statistical analysis was performed using the Wilcoxon test. $P < 0.05$ was considered significant.

RESULTS

In the awake state, only one patient of 14 was an oronasal breather; the others were nasal breathers. After administration of thiopentone, all the patients became drowsy, but responded to physical stimulation and verbal commands while maintaining airway patency (conscious sedation). During this sedated state, 11 of 13 patients who had been designated nasal breathers in the awake breathers continued to breathe only through the nasal route, while two nasal breathers started to breathe through both the nasal and oral routes. The patient designated as an oronasal breather in the awake state continued this pattern after sedation.

Figure 1 shows the responses to nasal occlusion observed in one of the nasal breathers. In this particular patient, during full wakefulness (fig. 1) nasal occlusion caused immediate cessation of air-flow, as shown by the nasal and oral carbon dioxide tracings. Oral breathing was initiated some 4 s after occlusion of the nasal passage, and there was almost no change in Sp_{O_2} . After the release of nasal occlusion, exclusive nasal breathing was resumed promptly.

After sedation (fig. 1) with thiopentone, there was a progressive increase in inspiratory efforts during nasal occlusion, but the onset of oral breathing was greatly delayed, leading to a progressive decrease in Sp_{O_2} . In two nasal breathers, nasal occlusion failed to initiate oral breathing within 60 s after occlusion (fig. 2), after which time they became hypoxic and the nasal occlusion was released. In these patients the response time was recorded as 60 s, although it was actually longer.

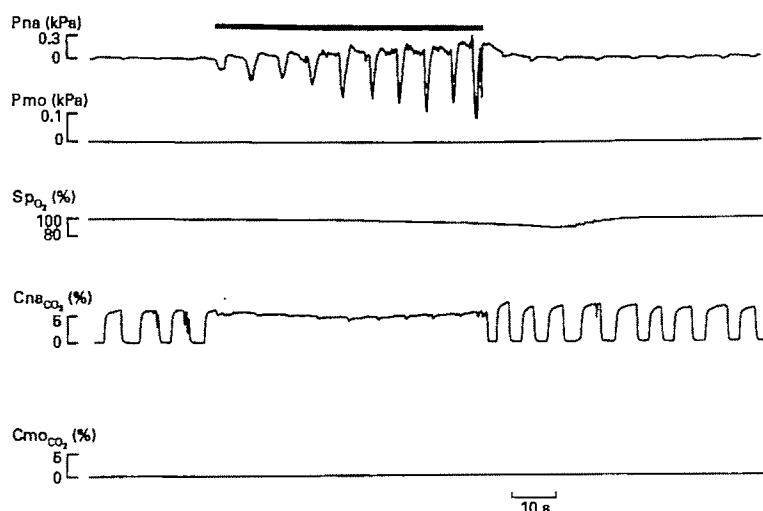


FIG. 2. Prolonged airway obstruction in response to nasal occlusion during sedation. Note that no initiation of oral breathing occurred during nasal occlusion. See figure 1 for abbreviations.

TABLE I. Ventilatory frequency (f), end-tidal PCO_2 (PE'_{CO_2}), and SpO_2 during the conscious state and sedation (mean (SD) [range]). ** $P < 0.01$ compared with conscious state and with before nasal occlusion

	Awake state	Sedation
f (b.p.m.)	14.5 (1.9) [10.4–18.0]	15.4 (2.3) [10.0–18.0] ($n = 14$)
PE'_{CO_2} (kPa)	5.3 (0.52) [4.7–6.5]	5.6 (0.51) [4.7–6.5] ($n = 14$)
SpO_2 (%)		
Before nasal occlusion	98.2 (0.6) [96–99]	97.3 (0.9) [96–99] ($n = 11$)
During nasal occlusion	98.0 (0.8) [97–99]	89.3 (4.3)** [82–95] ($n = 11$)

The mean response time in the conscious state obtained from 13 nasal breathers was 3.0 (1.2) s (range 1.8–6.3 s). In 11 nasal breathers who continued to breathe through the nose after administration of thiopentone, the response time during sedation (mean 37.7 (15.5) s; range 16–60 s) was significantly longer than that in the awake state (mean 3.2 (1.3) s; range 1.9–6.3 s) ($P < 0.01$). Also, there was a significant difference in the smallest values of SpO_2 attained during nasal occlusion between the awake and sedated states (table I).

DISCUSSION

Confirming observations made elsewhere [3–5], we have demonstrated that the majority of awake patients were nasal breathers during quiet breathing. Although the mechanisms and factors responsible for a marked preference of breathing via the nose are not entirely clear, both physiological and anatomical factors may influence the choice of breathing route.

Rodenstein and Stanescu [3] demonstrated that, when the mouth is open, the airflow depends on the position and function of the soft palate. Thus when

the soft palate lies downward against the base of the tongue, flow is entirely nasal. In contrast, when the soft palate extends horizontally from hard palate to posterior pharyngeal wall and closes the nasopharynx, air flows through mouth only. When the soft palate lies midway between tongue and posterior pharyngeal wall, air flows through both the nose and mouth. Two major muscles responsible for the active movement of the soft palate are the palatoglossus muscle, which directs the soft palate caudally and ventrally, and the levator veli palatini, which pulls the soft palate cephalad and in a dorsal direction.

The major finding in the present study was that, in nasal breathers, the ability to switch from nasal breathing to oral breathing in response to nasal occlusion was greatly obtunded during sedation with thiopentone. This finding is in good agreement with that of Wood and Harding [2], who showed, in lambs and ewes, that sedation with pentobarbitone and diazepam delayed the onset of oral breathing and led to a greater degree of asphyxia during nasal obstruction. However, considerable differences exist between sheep and humans in responses to nasal occlusion. For example, the time required to initiate oral breathing after nasal occlusion in awake humans obtained in this study (3.0 (1.2) s) is much shorter than that reported in awake ewes (59.7 (8.7) s) [2]. Also, a considerable decrease in SpO_2 was observed before initiation of oral breathing in awake ewes, whereas hypoxaemia was never observed in awake humans. These differences in responses to nasal occlusion between human and sheep may be explained by the difference in voluntary control of breathing route. Presumably, in humans the contribution of behavioural control may play a more important role in the change of breathing route than in sheep. Most subjects in this study reported that they were aware of nasal occlusion during wakefulness and might respond consciously to the occlusion.

The finding that sedation caused delayed onset of oral breathing after nasal occlusion supports our hypothesis that, in humans, wakefulness is the most

important factor in control of the change in breathing route. This view is also compatible with the observation of Rodenstein, Perlmutter and Stanescu [6] that in human infants the time required to initiate oral breathing after nasal occlusion is related to age, conscious state, or both; that is, infants who are older or awake, or both, respond faster than those who are younger or sleeping, or both. Similarly, Kuna and Smickley [7] observed in normal sleeping adults that, during non-REM sleep, oral breathing occurred only in association with arousal.

Other factors which may affect the control of the route of breathing are chemoreceptor and mechanoreceptor inputs. Although there is evidence [8, 9] that both mechanical and chemical sensory mechanisms are involved in the reflex initiation of oral breathing after nasal obstruction in experimental animals, the relative contribution of each of these factors to the control of oral breathing was not explored systematically in this study. However, an immediate initiation of oral breathing after nasal occlusion in the awake state suggests that at least chemical inputs play little or no role in control of breathing route in awake nasal breathers. In a previous study [10], we showed that, in awake subjects, topical lignocaine in the nasal passages delayed the onset of oral breathing in response to nasal occlusion (before lignocaine 4.4 (2.7) s; after lignocaine 10.8 (7.8) s), suggesting that sensory information arising from upper airway receptors may play an important role in controlling the change in breathing route. The greater differences found between full wakefulness and sedation in our study, compared with before and after topical upper airway anaesthesia, emphasize the importance of behavioural factors.

Whatever the mechanisms may be, some form of centrally co-ordinated series of motions such as opening the mouth, contracting the muscles of the tongue and elevation of the soft palate is necessary to open the oral passage and initiate oral breathing. In our study, because the mouth was kept open, contraction of the muscles of the tongue and elevation of the soft palate may have been two major factors necessary to create the oral airway. As adequate airway patency was maintained before nasal occlusion, even during sedation, it is possible that the delay in creating an oral airway during sedation might be caused mainly by preferential depression of activity in the genioglossus and levator veli palatini, leaving other airway-maintained muscles unaffected.

It should be noted that we studied only young female patients, and our findings may not be applicable to males or more elderly patients. However, as the control of the upper airway in males and elderly subjects is more capricious [11], it is possible that impairment of the ability to change the breathing route during sedation may be particularly pronounced in these populations.

Our findings may be relevant to the clinical problem of airway obstruction during sedation. Nasal obstruction could occur as a result of increased nasal secretory activity, increased mucosal congestion or the presence of a nasogastric tube. Under such circumstances, impairment of the ability to

initiate oral breathing could cause airway obstruction, despite apparent oral airway patency. It has been reported that, in sedated human adults, nasal packing for control of epistaxis causes hypoxaemia [12, 13].

Similar considerations may apply to patients with sleep apnoea. Indeed, several reports [14–16] suggest that nasal obstruction is an important predisposing factor in sleep apnoea. Release of airway obstruction during sleep in sleep apnoea patients often coincides with central arousal [17]. Although this release of oropharyngeal occlusion is considered to be the likely mechanism during central arousal, release of airway obstruction may result partly from change in breathing route from the nose to the mouth.

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PROLONGED PARALYSIS AFTER LONG-TERM, HIGH-DOSE INFUSION OF PANCURONIUM IN ANAESTHETIZED CATS

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SUMMARY

We have studied the neuromuscular effects of a 48-h infusion of high-dose pancuronium ($400 \mu\text{g kg}^{-1} \text{h}^{-1}$) in four cats anaesthetized with pentobarbitone, using contraction of tibialis anterior muscles after direct and indirect stimulation. After cessation of the pancuronium infusion, prolonged paralysis existed. The first twitch in the train-of-four stimuli (TOF) reappeared 8–12 h after termination of the pancuronium infusion. Twenty-four hours after termination of the infusion, TOF ratios were less than 0.08 and twitch contraction averaged 39 (SE 8) % of initial values. Twitch contraction after direct stimulation did not differ from initial values. Antagonism of paralysis was accomplished with neostigmine $60 \mu\text{g kg}^{-1}$ in two animals and neostigmine $90 \mu\text{g kg}^{-1}$ and 4-aminopyridine $500 \mu\text{g kg}^{-1}$ in the others. Steady-state plasma concentration of pancuronium (2000 ng ml^{-1}) decreased rapidly after termination of the infusion, but then stabilized at about 130 ng ml^{-1} . These results indicate that prolonged paralysis after long-term administration of high-dose pancuronium is caused primarily by failure of neuromuscular transmission, most likely caused by the persistent plasma concentrations of the drug in the pharmacologically active range. (Br. J. Anaesth. 1993; 71: 393–397)

KEY WORDS

Neuromuscular relaxants: pancuronium. Pharmacokinetics.

In the intensive care unit (ICU), neuromuscular blocking agents are used in doses and for periods of time far exceeding those used in the operating room [1]. Their long-term administration to ICU patients is associated with the development of prolonged peripheral muscle paralysis [2–6]. However, it is not clear if alterations in the pharmacokinetics of neuromuscular blockers or in neuromuscular transmission secondary to “pharmacological denervation” (possibly related to absolute or relative overdosage [2]) are responsible for this prolonged paralysis. As the existence of the post-treatment paralysis syndrome is clinically well documented for pancuronium [1, 6], we developed an experimental animal model in an attempt to resemble the conditions in the ICU. With this model we studied the

effects of prolonged administration of high-dose pancuronium on neuromuscular function and its pharmacokinetics.

MATERIALS AND METHODS

Anaesthesia

After obtaining institutional approval, we studied four adult male cats ($3.7\text{--}5.9 \text{ kg}$). Anaesthesia was induced with pentobarbitone $40 \text{ mg kg}^{-1} \text{ i.p.}$ (Hospital Pharmacy, Groningen). Anaesthesia was maintained for 72 h by a continuous infusion at an initial rate of $1 \text{ mg kg}^{-1} \text{ h}^{-1}$ administered through an i.v. cannula inserted in a cephalic vein. The trachea was intubated and artificial ventilation maintained with room air delivered by a V5KG-Narco-Biosystems Inc. ventilator, using a frequency of 20 b.p.m. and tidal volume of 15 ml kg^{-1} to maintain end-tidal PCO_2 in the range $4.0\text{--}4.5 \text{ kPa}$. Arterial pressure and heart rate were recorded continuously (MT 9500 Astro Med) via a cannula inserted in the carotid artery. The urinary bladder was cannulated and urine output measured. The core temperature was kept at $38 \pm 0.5^\circ\text{C}$ using a heating pad and controlled by a temperature probe placed in the oesophagus. To maintain fluid balance, glucose in 0.45 % saline and Haemacel were administered by infusion pumps (IVAC) to maintain a urinary output of approximately $3 \text{ ml kg}^{-1} \text{ h}^{-1}$. The rate of pentobarbitone infusion was dictated by arterial pressure and heart rate. A central venous infusion of dopamine hydrochloride (Hospital Pharmacy, Groningen) was started at an initial rate of $1 \mu\text{g kg}^{-1} \text{ min}^{-1}$, if mean arterial pressure decreased to less than 80 mm Hg.

Antibiotic regimen

Selective gut decontamination and systemic antibiotic prophylaxis were used to prevent infection. Drinking water was replaced by a solution containing tobramycin 20 mg, colistin 100 mg and amphotericin B 200 mg dissolved in 100 ml, 24 h before the start of

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the experiment. Animals consumed at least 75 ml of this solution. Antibiotic prophylaxis was provided by cefotaxime 25 mg kg⁻¹ i.v. administered every 12 h during the first 60 h of the study.

Neuromuscular function

Neuromuscular function was measured using the common peroneal nerve-tibialis anterior muscle preparation of both legs. Contractions were obtained after 15-min periods of stabilization. Silver wire electrodes were placed on the nerves. The tendons of both tibialis anterior muscles were freed, together with a piece of bone, and connected to identical force displacement transducers (Maryland Instruments Ltd). Indirect muscle contractions were elicited by supramaximal square wave stimuli of 0.2 ms duration, either as single twitches (0.1 Hz) or train-of-four (TOF) (2 Hz for 2 s every 12 s) and recorded on a polygraph. Direct stimulation was elicited by supramaximal stimuli of 2 ms duration (0.1 Hz) applied via an electrode placed between the patella and lateral condyle and a second electrode inserted on the distal tendon of the muscle.

After initial recordings, the muscle tendons were sutured in their original positions and skin was closed to prevent infection. During the study, each muscle was stimulated differently. The stimulating electrodes on the right peroneal nerve were removed for the duration of the study and the fasciae and skin were closed. The left peroneal nerve was stimulated at a frequency of 1 TOF min⁻¹ for the duration of the pancuronium infusion (48 h). For the remaining 24 h until the end of the study, TOF stimuli were applied every 15 min for 2 min. Muscle response was determined by visual and tactile assessment. At the end of the study, electrodes were replaced on the right peroneal nerve and the muscle tendons were freed and connected to the transducers again.

Neuromuscular block was induced by a single bolus dose of pancuronium 600 µg kg⁻¹ (Organon Teknika), followed by an i.v. infusion of pancuronium 400 µg kg⁻¹ h⁻¹ for 48 h. Residual paralysis present at the end of the study (72 h) was antagonized until fade in TOF was abolished as follows. Initial antagonism was attempted with neostigmine 30 µg kg⁻¹, which was repeated twice if recovery of fade remained incomplete. If TOF fade persisted after three doses of neostigmine, 4-aminopyridine 500 µg kg⁻¹ (Hospital Pharmacy, Groningen) was administered.

Sampling and analysis

During the pancuronium infusion, samples of blood and urine were obtained every 8 h; thereafter, samples of blood were obtained every 4 h, whereas urine was sampled every 8 h. The volume of each blood sample withdrawn (2 ml) was replaced by an equal amount of Haemaccel. At the end of the experiment one bile sample (≥ 8 ml), the liver, kidneys, lungs and tibialis anterior muscles, and some cartilage tissue were removed and weighed. Blood and urine samples and tissue specimens were stored at -18 °C until required for chemical analysis.

The concentrations of pancuronium and its puta-

tive metabolites, 3-desacetyl, 17-desacetyl and 3, 17-desacetyl pancuronium, were determined by high pressure liquid chromatography (HPLC) and post-column ion pair extraction with fluorimetric detection, according to the method described for vecuronium [7].

Statistical analysis

Because of the small number of experiments, most data are presented without summary statistics, otherwise as mean (SE). Statistical analysis was performed by paired and unpaired Student's *t* test or the Mann-Whitney *U* test as indicated. Differences were considered significant at *P* < 0.05. Analysis of pharmacokinetic data was performed with RUGFIT [8], a computer program using iterative non-linear regression analysis.

RESULTS

Experimental conditions

Anaesthesia and prolonged artificial ventilation were tolerated well for 72 h by the four animals and no signs of infection or changes in end-tidal carbon dioxide partial pressure, temperature or urinary output were observed. The rate of infusion of pentobarbitone was reduced from 1 mg kg⁻¹ h⁻¹ to approximately 20 % of the initial infusion rate in the course of the study. Maintenance of mean arterial pressure greater than 80 mm Hg required the infusion of dopamine in three animals (cat No. 1 during the period 36–62 h (2.6 (0.7) µg kg⁻¹ min⁻¹); cat No. 3: 26–48 h (2.5 (0.5) µg kg⁻¹ min⁻¹); cat No. 4: 26–30 h (4.5 (2.1) µg kg⁻¹ min⁻¹). Mean arterial pressure and heart rate increased significantly after cessation of the infusion of pancuronium (fig. 1) (MAP from 89.4 (2.1) mm Hg to 114.4 (3.3) mm Hg (*P* < 0.01; Mann-Whitney *U* test); heart rate from 135 (12) beat min⁻¹ to 170 (13) beat min⁻¹ (*P* < 0.05; Mann-Whitney *U* test) during the 4 h before and 4 h after termination of the infusion of pancuronium).

Neuromuscular function

The first twitch contractions in response to indirect TOF stimulation of the left tibialis anterior muscle could be observed about 10 h after the discontinuation of the pancuronium infusion (cats

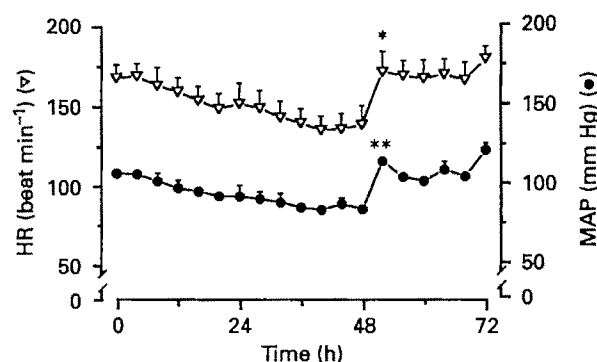


FIG. 1. Mean arterial pressure (MAP) (●) and heart rate (HR) (▽) during the study (mean, SE). After an initial bolus dose of 600 µg kg⁻¹, pancuronium was infused at 400 µg kg⁻¹ h⁻¹ during 48 h. Significant differences from previous period: **P* < 0.05; ***P* < 0.01 (Mann-Whitney *U* test).

Nos 1-4: 8 h, 12 h, 12 h and 9 h, respectively). At the end of the study (24 h after termination of the pancuronium infusion) muscle paralysis was still present: TOF ratios were 0.08 or less, while the twitch tension evoked by indirect stimulation averaged about 40% of values observed at the start of the study (table I). Complete abolition of fade in the TOF response was accomplished with two doses of neostigmine (total: 60 $\mu\text{g kg}^{-1}$) in cats Nos 1 and 2. In the other two animals, TOF ratio was 0.81 (0.05) ($n = 4$) after three doses of neostigmine (total dose 90 $\mu\text{g kg}^{-1}$). Further antagonism of neuromuscular block in these two animals was attempted by increasing the acetylcholine concentration by stimulating its release from the nerve ending by administration of 4-aminopyridine 500 $\mu\text{g kg}^{-1}$; this abolished TOF fade, but also augmented slightly the first twitch in the TOF, by 10.2 (2.7)% ($n = 4$). After complete reversal of TOF fade, indirectly evoked twitch tensions had doubled, on average, to about 84% of initial values (table I). There were no explicit changes in directly evoked muscle contraction, assessed before antagonism of block, compared with initial values (table II).

Plasma concentrations

The steady-state plasma concentration of pancuronium observed during continuous infusion decreased rapidly after termination of the infusion and stabilized at approximately 130 ng ml^{-1} , with only an extremely slow further decline until the end of the study (fig. 2). Plasma concentrations of 3-desacetyl pancuronium were measurable in only two animals. The plasma concentration curves of this metabolite followed a pattern similar to that of the parent compound, but at considerably smaller concentrations, approximating 20 ng ml^{-1} during the plateau phase at the end of the study (fig. 2). During processing, the 48-h sample from one study was lost (cat No. 1). In the remaining three animals, the plasma concentration decay curve of pancuronium was described best by a bi-exponential equation. The half-life of the first component of the curve, estimated by computer fitting, was 87 min, 41 min and 61 min in cats Nos 2, 3 and 4, respectively. The half-life of the second component could not be determined reliably, but was estimated to be longer than 3 days. The volume of distribution of pancuronium at steady state (V^{ss}) was calculated as the quotient of the body content and the plasma concentration at 48 h. Body content of pancuronium (10.3 (0.3) mg kg^{-1} ; $n = 4$) was estimated assuming exclusive renal excretion of the drug—9.5 (0.3) mg kg^{-1} at 48 h. Hence V^{ss} was estimated as 5.2 (0.7) litre kg^{-1} .

The recovered amounts of pancuronium and two of its desacetyl derivatives are summarized in table III. The total amounts of unchanged pancuronium in urine, liver and bile were about 56%, 11% and 11% of the administered dose, respectively. Two metabolites, the 3-desacetyl and the 17-desacetyl derivatives, were also detected in the urine, bile, liver and kidneys, but in negligible amounts. The total recovery of unchanged pancuronium in this study was about 82% of the administered dose.

TABLE I. Post-treatment indirectly stimulated twitch tension before and after antagonism by neostigmine and 4-aminopyridine (4-AP), expressed as percentage of control values, for the stimulated (left) and unstimulated (right) side in individual cats

Cat No.	Antagonist	Before antagonism		After antagonism	
		Left (%)	Right (%)	Left (%)	Right (%)
1	Neostigmine 60 $\mu\text{g kg}^{-1}$	31.5	36.5	60.5	87.5
2	Neostigmine 60 $\mu\text{g kg}^{-1}$	66.2	41.2	82.7	50.9
3	Neostigmine 90 $\mu\text{g kg}^{-1}$	60.8	24.6	104.9	79.3
4	Neostigmine 90 $\mu\text{g kg}^{-1}$ + 4-AP 500 $\mu\text{g kg}^{-1}$	32.0	19.7	100.0	103.8

TABLE II. Post-treatment twitch tension as percentage of the control values on direct stimulation

Cat No.	Left (%)	Right (%)
1	116	93
2	80	63
3	93	65
4	100	100

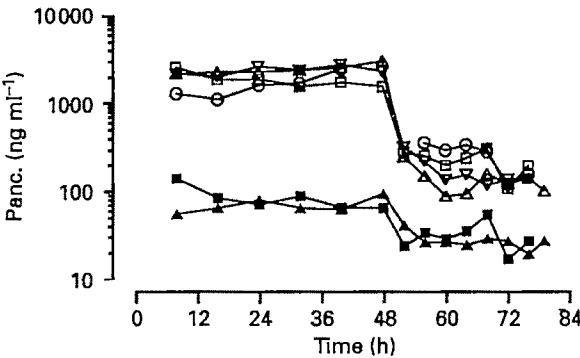


FIG. 2. Plasma concentration of pancuronium (open symbols) and its 3-desacetyl derivative (filled symbols) in individual cats. After an initial bolus dose of 600 $\mu\text{g kg}^{-1}$, pancuronium was infused at 400 $\mu\text{g kg}^{-1} \text{ h}^{-1}$ during 48 h. 3-Desacetyl pancuronium was detected in only two animals.

TABLE III. Percentage of the total dose (19800 $\mu\text{g kg}^{-1}$) recovered at the end of the study as unchanged pancuronium and its 3-desacetyl and 17-desacetyl derivatives from urine, bile and various organs (mean (SE); $n = 4$). Recovery in muscle was based on an estimated muscle weight of 40% of total body weight

Source	Pancuronium (%)	3-Desacetyl pancuronium (%)	17-Desacetyl pancuronium (%)	Total recovered (%)
Urine	53.6 (3.1)	2.1 (0.5)	—	55.6 (3.0)
Liver	8.0 (0.8)	2.7 (0.3)	—	10.9 (1.1)
Bile	8.5 (1.0)	2.0 (0.4)	0.4 (0.1)	10.9 (1.5)
Muscle	3.6 (1.6)	—	—	3.6 (1.6)
Kidney	0.5 (0.2)	0.1 (0.1)	—	0.6 (0.2)
Lung	0.2 (0.1)	—	—	0.2 (0.1)
Cartilage	0.1 (0.1)	—	—	0.1 (0.1)
Total	74.5 (3.4)	6.9 (0.7)	0.4 (0.1)	81.6 (4.0)

DISCUSSION

In three pilot studies (not described here) we observed that infection, probably through the open wounds of the nerve-muscle preparation, is un-

avoidable in spite of antibiotic prophylaxis. For this reason we closed the wounds after the initial measurements and resumed quantitative neuromuscular monitoring 24 h after termination of the pancuronium infusion. The prophylactic antibiotic regimen was designed to exclude possible effects on neuromuscular function or the pharmacokinetics of pancuronium. Selective gut decontamination by non-absorbable antibiotics was used [9]. To provide systemic antibiotic prophylaxis a cephalosporin (cefotaxime) was chosen, as this group of antibiotics does not influence neuromuscular function [9, 10].

The conditions of the study were satisfactory as reflected by the haemodynamic status, blood-gas values and renal function. During the infusion of pancuronium, there was a decrease in heart rate and mean arterial pressure, but rapid restoration of these variables to control values after termination of the pancuronium infusion (fig. 1), suggesting a drug-related effect. The probable mechanism is ganglion block, which occurs at both sympathetic and parasympathetic ganglia. As the circulatory system of anaesthetized animals is usually under increased sympathetic tone [11, 12], block of nicotinic receptors in autonomic ganglia would be expected to decrease mean arterial pressure and heart rate. Although pancuronium does not produce ganglion block in doses which provide neuromuscular block [12], very large bolus doses of the agent have been reported to produce ganglion block in the cat [13]. Additional effects on sympathetic nerves that may account for the circulatory effects of large concentrations of pancuronium are the depletion of noradrenaline in nerve terminals because of inhibition of re-uptake [14, 15] and the decrease of noradrenaline release as a result of block of presynaptic muscarinic receptors [16].

After cessation of the 48-h pancuronium infusion, spontaneous recovery of muscle function was protracted, as demonstrated by the appearance of the first twitch of the TOF response only 10 h after termination of the pancuronium infusion. At the end of the study, 24 h after termination of the infusion, TOF fade was marked and single twitch tension decreased compared with values obtained at the start of the study. This prolonged paralysis was probably caused by failure in neuromuscular transmission, rather than decline in skeletal muscle function, in view of the less obvious decrease in muscle contraction evoked by direct stimulation. This is supported further by the observation that repeated administration of neostigmine largely restored TOF response and increased single twitch tension. An additional recovery in TOF response and single twitch tension was observed in the two animals in which neostigmine incompletely antagonized TOF fade response after administration of 4-aminopyridine. The action of 4-aminopyridine might be explained by its action on nerve endings, enhancing acetylcholine release, but also by direct stimulatory effects of the compound on muscle contraction [17].

The pharmacokinetics of pancuronium after long-term administration differed from its bolus dose kinetics. After termination of a steady-state infusion, one would expect the plasma concentration curve to

follow a decay curve characterized by the same half-lives as after single bolus injection. However, after prolonged administration, the relatively rapid decrease in plasma pancuronium concentration after termination of the infusion was followed by a plateau phase from which decline was so slow that no reliable half-life could be determined (> 72 h). Although the estimate of initial half-lives of the plasma concentration decay curves (41–96 min) may not be very accurate because of too few sampling points, these half-lives are in the same range as the elimination half-life (32 min) observed after a single bolus injection of pancuronium in the cat [18]. The extremely prolonged terminal half-life (> 72 h) observed in this study was accompanied by an enlarged volume of distribution. As a considerable amount of pancuronium was recovered from the liver (2.2 mg kg^{-1}), this organ might serve as the principal store. Pancuronium has been shown to be stored preferentially in the mitochondrial fraction of isolated rat liver and to be excreted in bile less efficiently than other neuromuscular blocking agents [19]. The supposition that the hepatic uptake of pancuronium represents a deep compartment responsible for the large volume of distribution is supported by a study in rats [20], which showed the presence of radiolabelled pancuronium in the liver up to 1 month after i.p. injection of a single bolus of 0.3 mg kg^{-1} .

In agreement with earlier studies [18, 20] the results of the present study show that pancuronium is excreted mostly unchanged in the urine and bile. In addition, considerable amounts were recovered from the liver. The extent of biotransformation of pancuronium was limited and of the same order of magnitude as reported previously [18, 20]. Total recovery of pancuronium and its metabolites was about 82 % of the administered dose—similar to the total recovery of 84 % and 80 % reported by other authors [18, 20].

It is unclear to what extent the persistent plasma concentrations of pancuronium contributed to the prolonged paralysis observed in this study, as no concentration–effect relationship for pancuronium has been described in the cat. However, human data with pancuronium [21] suggest that the above concentrations are pharmacologically active. There are few kinetic data of patients treated for prolonged periods of time with large doses of pancuronium, but the existence of a deep compartment may be anticipated and is supported by a case report of pancuronium overdose [22] in a patient with renal failure who had received about pancuronium 15 mg kg^{-1} during 4 days and retained pharmacologically active plasma concentrations of pancuronium and 3-desacetyl pancuronium for several days after administration, despite continuous haemofiltration and intermittent dialysis. Moreover, large amounts of pancuronium and its 3-desacetyl metabolite were recovered from the liver and muscle of this patient 6 days after infusion, at postmortem examination [22]. Existence of a deep compartment for pancuronium in man, however, does not exclude its use *per se* in ICU patients, as the plasma concentration of the associated plateau phase is pro-

portional to the administered dose. Therefore, this pharmacokinetic complication may be attenuated if administration of the drug is guided by adequate neuromuscular monitoring.

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SOLUBILITY CHARACTERISTICS OF THE IDEAL AGENT FOR MEASUREMENT OF CARDIAC OUTPUT BY SOLUBLE GAS UPTAKE METHODS

R. R. KENNEDY AND A. B. BAKER

SUMMARY

Measurements of the rate of uptake of soluble gases such as nitrous oxide or acetylene has been used as the basis of a method of cardiac output estimation since 1912. Using theoretical models of single, multiple and rebreathing techniques of cardiac output measurement, we determined the effect of solubility in blood and lung tissue on the changes in the final expired concentration of the gas. Decreasing lung tissue solubility increased the sensitivity of all three models to cardiac output changes. When the lung tissue/blood partition coefficient was 1, the optimum blood/gas partition coefficients were 2.6 for the single-breath model, 4.1 for the three breath-model and 3.5 for the rebreathing model. A selection of gases, including volatile anaesthetic agents was studied using the same models. Under most conditions, enflurane approximated most closely to the requirements for an ideal agent for use in this technique of cardiac output measurement. (Br. J. Anaesth. 1993; 71: 398–402)

KEY WORDS

Heart: cardiac output. Measurement techniques: cardiac output.

The rate of uptake of soluble gases from the lungs may be used to quantify cardiac output. Many gases have been used for this purpose, including oxygen, carbon dioxide, acetylene and nitrous oxide. The techniques have been reviewed comprehensively by Sackner [1].

The use of non-physiological gases (that is, not oxygen, carbon dioxide or nitrogen) for this purpose has the particular advantage that the venous concentration may be assumed to be zero, provided all measurements are completed before recirculation occurs. In man, without significant cardiovascular disease, recirculation occurs after 14–18 s at rest and after 8 s during heavy exercise [2, 3]. In general, the agents used are those which anaesthetists consider relatively insoluble, with blood/gas partition coefficients (λ_{bl}/g) less than 1, such as nitrous oxide [2, 4] ($\lambda_{bl}/g = 0.47$) and acetylene [5–7] ($\lambda_{bl}/g = 0.74$). Physiologists frequently describe such gases as soluble, in contrast to the “insoluble gases” such as helium and sulphahexafluoride.

Assuming uptake is perfusion-limited, the rate of

uptake of any agent is proportional to the solubility in blood of that agent. Therefore, a more soluble agent is taken up initially at a greater rate than a less soluble gas, producing a larger reduction in alveolar concentration which should be easier to measure.

Studies with ether [4, 8] ($\lambda_{bl}/g = 13$) and acetone [4] suggest they are “too soluble”, particularly in the mucosa of the airway and other parts of the ventilatory tract not forming part of the gas exchange space. These gases diffuse into these tissues during inspiration, but only a small amount is removed by blood flow; most of the gas remains in the tissue and is released into the airway during expiration, contaminating the expiratory alveolar gas sample. This “extra-alveolar” uptake does not appear to be a problem with relatively insoluble agents such as nitrous oxide and acetylene [4]. Recent theoretical [9] and experimental [5] work also suggested there is more variability in results obtained using ether than when acetylene is used. Agents with solubilities between those of acetylene and ether have not been studied previously.

We hypothesized that modern volatile anaesthetic agents such as halothane ($\lambda_{bl}/g = 2.4$), enflurane ($\lambda_{bl}/g = 1.9$) and isoflurane ($\lambda_{bl}/g = 1.5$) might fall in the middle ground, with greater blood solubility producing a useful increase in concentration change, whilst not being so soluble as to contaminate end-tidal samples.

The aim of this study was to determine the conditions which produced the greatest change in expired concentration (F_A) as a fraction of inspired concentration (F_I) (F_A/F_I) for a given change in cardiac output. We also wanted to rank different soluble gases, and particularly volatile anaesthetics, in the same way, to determine which are the most sensitive to cardiac output changes. The second part of our hypothesis, which considers the effect of extra-alveolar solubility, required experimental work and is not part of the studies reported here.

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METHODS

To describe the relationship between cardiac output and expired concentration, we used three mathematical models representing different ventilatory manoeuvres: a single breath; three breaths, with the same inspired concentration for each breath; and rebreathing with the inspired concentration for each of seven breaths determined by the contents of the rebreathing bag.

The relationship between the rate of uptake and cardiac output (taking cardiac output ($\dot{Q}$) to be equal to the effective pulmonary blood flow) is described by the Fick equation [10,11] which states that, for a substance Z :

$$\begin{aligned} \text{rate of uptake of } Z \\ = \dot{Q} \cdot [\text{arterial} - \text{venous content of } Z] \end{aligned}$$

which may be written as:

$$\text{rate of uptake of } Z = \dot{Q} \cdot \lambda_{bl/g} \cdot [F_a - F_v]$$

where $\lambda_{bl/g}$ = the blood/gas partition coefficient; F_a and F_v = arterial and venous partial pressures, respectively, of Z (expressed as fractions of ambient atmospheric pressure). If uptake is not diffusion-limited, $F_a = F_A$, where F_A = fractional alveolar concentration.

During a breath-holding period and while $F_v = 0$, it can be shown [4, 12] (using the abbreviations listed in the Appendix) that:

$$F_A = F_{A_0} \cdot \exp - [\lambda_{bl/g} \cdot \dot{Q} \cdot t / V_A^{\text{eff}}] \quad (1)$$

where t = time since the end of inspiration; F_{A_0} = concentration at time $t = 0$; V_A^{eff} = effective alveolar volume, assuming perfect instantaneous mixing into homogeneous alveoli and constant alveolar volume.

After the initial inspiration, F_{A_0} is given by:

$$F_{A_0} = F_I \cdot (V_T - V_D) / V_A^{\text{eff}} \quad (2)$$

Combining these equations gives:

$$F_A = F_I \cdot [(V_T - V_D) / V_A^{\text{eff}}] \cdot \exp - [\lambda_{bl/g} \cdot \dot{Q} \cdot t / V_A^{\text{eff}}] \quad (3)$$

or, dividing both sides by F_I :

$$F_A / F_I = [(V_T - V_D) / V_A^{\text{eff}}] \cdot \exp - [\lambda_{bl/g} \cdot \dot{Q} \cdot t / V_A^{\text{eff}}] \quad (3a)$$

Many gases dissolve readily in the lung tissue itself and reach equilibrium in less than 2 s [13]. Thus the product of the lung tissue/gas partition coefficient ($\lambda_{lu/g}$) and the lung tissue volume (V_{lu}) represents

an extra "space" that acts as if it were part of the alveolar gas space [4, 14]. The lung tissue/gas partition coefficient is the product of the blood/gas and lung tissue/blood partition coefficients. Hence:

$$V_A^{\text{eff}} = V_{A_0} + \lambda_{bl/g} \cdot \lambda_{lu/bl} \cdot V_{lu} \quad (4)$$

or

$$V_A^{\text{eff}} = V_{A_0} + \lambda_{lu/g} \cdot V_{lu}$$

represents the total "volume of distribution" of the particular agent. Using the values for tissue solubilities shown in table I, if $V_{lu} = 0.5$ litre [4, 14] then, for halothane, $\lambda_{lu/g} \cdot V_{lu} = 2.4$ litre, while for nitrous oxide $\lambda_{lu/g} \cdot V_{lu} = 0.235$ litre. If the end-inspiratory alveolar gas space is 3.5 litre (using $V_A = 2.5$ litre and $V_T = 1$ litre), the total effective gas exchange space is 5.9 litre for halothane, but only 3.735 litre for nitrous oxide.

Equation (3) describes the single-breath model and is the basis for the three-breath and rebreathing models.

To allow for more than one breath, a simple model of ventilation [15] was used. In this model of ventilation, inspiration and expiration occur instantaneously and therefore the time-volume plot is a square wave. This model allows uptake over a series of ventilatory cycles to be considered as a sequence of "single breaths" using equation (3), but with different initial concentrations and lung volumes for each phase.

Concentrations in the lung were calculated at three times during each ventilatory cycle: at the end of inspiration (F_{AI}'); at the end of the inspiratory hold (F_{AE} —the actual end-expired concentration); and at the end of expiratory hold (F_{AE}' —the concentration in the lungs at the start of the next breath). F_{AE} and F_{AE}' were calculated by applying equation (1) using appropriate initial concentrations (F_{AI}' and F_{AE}), lung volumes ($V_A^{\text{eff}} + V_T$ for the inspiratory hold period and V_A^{eff} for the expiratory hold period) and times (t_{in} and t_{out}). End-inspired concentration (F_{AI}') was calculated as:

$$\begin{aligned} F_{AI}' = [F_{AE}' \cdot V_A^{\text{eff}} \\ + F_{AE}' \cdot V_D + F_I \cdot (V_T - V_D)] / [V_A^{\text{eff}} + V_T] \quad (5) \end{aligned}$$

where F_I and F_{AI}' are expressed as fractions of the initial F_I .

Equations (3) and (5) define the three-breath model, which can be extended to any number of breaths.

In the rebreathing model, the inspired concentration for each breath was the concentration in the rebreathing bag at the end of the previous expiration. This was calculated as:

$$\begin{aligned} F_{bag} = [F_I \cdot (V_{bag} - V_T) \\ + F_I \cdot V_D + F_{AE} \cdot (V_T - V_D)] / V_{bag} \quad (6) \end{aligned}$$

where $F_I = F_{bag}$ from the previous breath.

In addition to the assumptions common to all models, the rebreathing model assumes no additional deadspace (equipment deadspace = 0), no uptake of the agent onto the bag, and that the total volume of the system remains constant; that is, uptake is minimal compared with the total volume of the lung-bag system.

TABLE I. Specific agents studied and partition coefficients used. Values are those used by Lowe and Ernst [16], except for helium [4]

Agent	Partition coefficient		
	Blood/gas	Lung/blood	Lung/gas
Helium	0.0086	1.07	0.0092
Nitrous oxide	0.47	1.0	0.47
Acetylene	0.75	1.03	0.77
Isoflurane	1.5	1.8	2.7
Enflurane	1.9	1.3	2.5
Halothane	2.4	2.0	4.8
Methoxyflurane	11	2.1	23.1
Ether	13	1.0	13

Standard conditions listed in the Appendix were used. The single-breath model was studied for 15 s, while the three-breath and rebreathing models were studied at three breaths (12.5 s) and seven breaths (13 s), respectively. These values were chosen to be less than the recirculation time of man at rest (14–18 s) [2]. Values of the partition coefficients used are listed in table I. Results are expressed as end procedure expired concentration divided by initial F_I or F_A/F_I .

The three models described above calculate F_A/F_I after completion of the test manoeuvre and assume perfect instantaneous mixing of gas, with no ventilation perfusion abnormalities. Similar models have been shown to produce valid results in patients without significant lung pathology [6, 9].

We chose to look at the effects of a change in cardiac output from 1 litre min^{-1} to 10 litre min^{-1} and defined the difference in F_A/F_I between these values as:

$$\begin{aligned} F_A/F_{I(\dot{Q}_{1-10})} &= F_A/F_{I(\dot{Q}_{1 \text{ litre min}^{-1}})} \\ &\quad - F_A/F_{I(\dot{Q}_{10 \text{ litre min}^{-1}})} \end{aligned}$$

$F_A/F_{I(\dot{Q}_{1-10})}$ was calculated for a range of combinations of $\lambda_{\text{bl/g}}$ and $\lambda_{\text{lu/bl}}$ ($\lambda_{\text{bl/g}}$ (1–15), $\lambda_{\text{lu/bl}}$ (0–5)) using the single-breath, three-breath constant inspired concentration and rebreathing models. This was repeated for a selection of anaesthetic gases and vapours. For comparison, examples of very soluble (methoxyflurane) and very insoluble (helium) agents were included.

In addition, the difference in F_A/F_I produced by a 25% increase in cardiac output was calculated for initial values of cardiac output between 1 and 10 litre min^{-1} .

RESULTS

Families of curves for different values of $\lambda_{\text{lu/bl}}$ using a single breath of 15 s duration (fig. 1), the three-breath model (fig. 2) and the rebreathing model (fig. 3) show that optimum combinations of $\lambda_{\text{bl/g}}$ and $\lambda_{\text{lu/bl}}$ existed for each model. The maximum value on each curve represents that blood solubility which gave the greatest change in expired concentration for a change in cardiac output from 1 litre min^{-1} to 10 litre min^{-1} at that $\lambda_{\text{lu/bl}}$. These values increased slightly as $\lambda_{\text{lu/bl}}$ decreased.

Table II shows the change in end procedure F_A/F_I with a change in cardiac output from 1 litre min^{-1} to 10 litre min^{-1} for a variety of anaesthetic agents and helium. Enflurane was the most sensitive to changes in cardiac output in all models, although ether performed well in the three-breath model. The three-breath model produced the greatest changes in F_A/F_I for the cardiac output change studied, but these were only marginally greater than those seen with the rebreathing model.

Figure 4 illustrates the effect of a 25% increase in cardiac output on the change in alveolar concentration and demonstrates that enflurane produced the greatest change at almost all values modelled. With the exception of the very soluble agents, ether and methoxyflurane, the curves are nearly linear over the range studied.

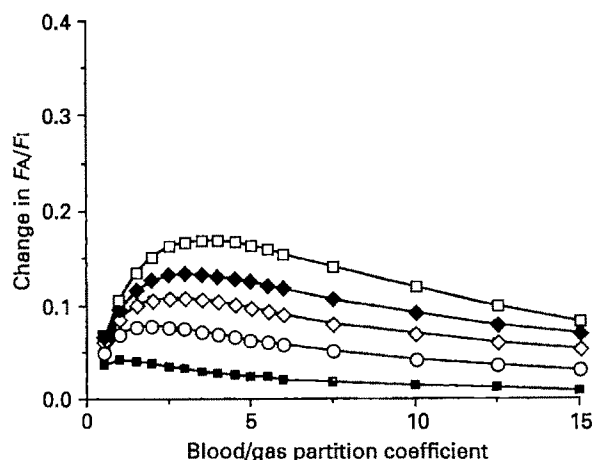


FIG. 1. Effect of lung and blood solubility on change in end procedure F_A/F_I for a change in cardiac output from 1 litre min^{-1} to 10 litre min^{-1} using the 15-s single-breath model. Initial conditions in all figures are as specified in the Appendix. Lung tissue/blood partition coefficients: $\square = 0$; $\blacklozenge = 0.5$; $\diamond = 1$; $\circ = 2$; $\blacksquare = 5$.

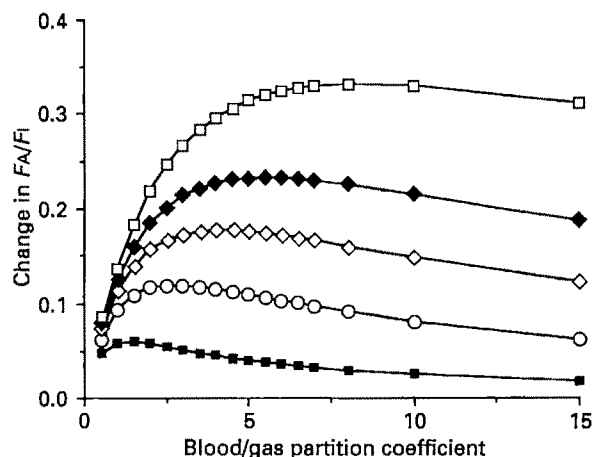


FIG. 2. Effect of lung and blood solubility on change in end procedure F_A/F_I for a change in cardiac output from 1 litre min^{-1} to 10 litre min^{-1} using the three-breath model. Lung tissue/blood partition coefficients: $\square = 0$; $\blacklozenge = 0.5$; $\diamond = 1$; $\circ = 2$; $\blacksquare = 5$.

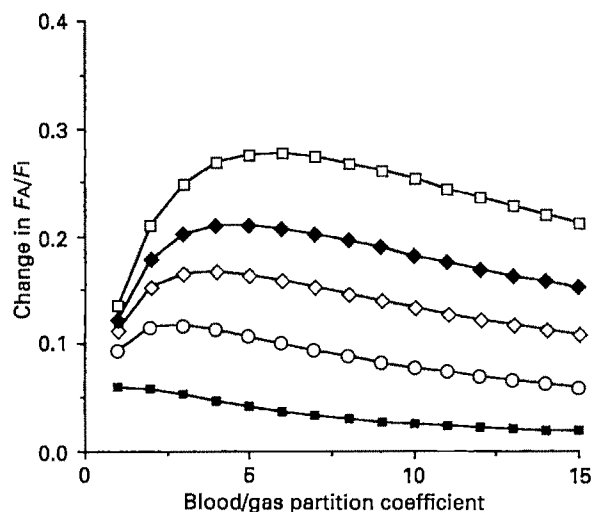


FIG. 3. Effect of lung and blood solubility on change in end procedure F_A/F_I for a change in cardiac output from 1 litre min^{-1} to 10 litre min^{-1} using the rebreathing model. Lung tissue/blood partition coefficients: $\square = 0$; $\blacklozenge = 0.5$; $\diamond = 1$; $\circ = 2$; $\blacksquare = 5$.

TABLE II. Effect of a change in cardiac output ($\dot{Q}$) from 1 litre min^{-1} to 10 litre min^{-1} for each of the models and selected agents, showing the end-procedure (Initial) FA/FI for $\dot{Q} = 1$ litre min^{-1} and the change in FA/FI when $\dot{Q}$ was increased to 10 litre min^{-1}

Agent	Single breath FA/FI		Three breath FA/FI		Rebreathing FA/FI	
	Initial	Change	Initial	Change	Initial	Change
Helium	0.243	0.0013	0.565	0.0009	0.428	0.0015
Nitrous oxide	0.221	0.054	0.530	0.067	0.400	0.067
Acetylene	0.208	0.073	0.510	0.093	0.383	0.093
Isoflurane	0.162	0.081	0.422	0.115	0.315	0.113
Enflurane	0.162	0.097	0.426	0.141	0.316	0.138
Halothane	0.130	0.078	0.354	0.119	0.263	0.117
Methoxyflurane	0.047	0.038	0.146	0.073	0.107	0.070
Ether	0.061	0.058	0.199	0.131	0.142	0.117

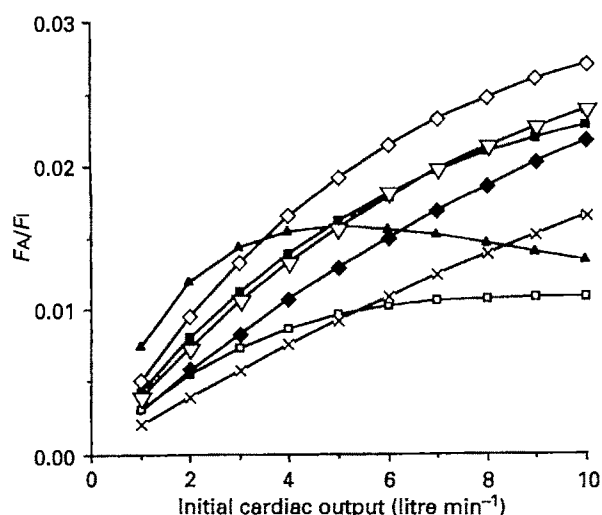


FIG. 4. Effect of a 25% increase in cardiac output from initial values between 1 and 10 litre min^{-1} on the change in end procedure FA/FI for a variety of anaesthetic agents. $\times$ = Nitrous oxide; $\diamond$ = acetylene; ∇ = isoflurane; $\diamond$ = enflurane; $\blacksquare$ = halothane; $\square$ = methoxyflurane; $\blacktriangle$ = ether.

The changes in FA/FI seen with isoflurane and halothane are very similar under all conditions studied and are consistently smaller than those with enflurane.

DISCUSSION

It is of interest that there were sets of optimum solubilities and that in general the "ideal" blood/gas partition coefficients were in the range 2–4, close to those of the modern volatile anaesthetic agents. For $\lambda_{lu}/bl = 1$, the maximum change in FA/FI was seen at a λ_{bl}/g of 2.6 with the single-breath model, 4.1 for the three-breath model and 3.5 for the rebreathing model. The effect of decreasing λ_{lu}/bl in any model was to increase the $FA/FI_{(Q1-10)}$ index and to increase the optimum λ_{bl}/g . A decrease in λ_{lu}/bl from 1 to 0 in the three-breath model had the effect of increasing the change in expired concentration by a factor of 1.8. However, none of the anaesthetic agents investigated had values of λ_{lu}/bl less than 1. The magnitude of the change in FA/FI varied between models, but was greatest with the three-breath model for all agents except helium (table II).

The rebreathing model produced results similar to those from the three-breath model. This was not

surprising because, given the same initial concentration, the only real difference between the two models is that the total mass of drug delivered to the alveolar gas space is greater with the three-breath model.

From table II it is apparent that there are considerable differences between agents with similar blood solubility, such as isoflurane and enflurane. We were initially surprised that enflurane, with a smaller partition coefficient, produced a greater change than that seen for halothane. The pattern of results may be explained by considering the physical process being modelled. For small values of λ_{bl}/g (nitrous oxide) the rate of uptake is small and FA/FA_0 is close to 1 (equation (1)). If cardiac output doubles, the decrease is, at most, double, but FA/FA_0 is still close to 1. Conversely, if λ_{bl}/g is large (for example ether), uptake is rapid and almost complete resulting in a small value for FA/FA_0 . In this case doubling cardiac output has little added effect, as FA/FA_0 (and FA/FI) is already close to zero. Between these extremes lies an optimum value for λ_{bl}/g with which a change in cardiac output produces a usefully large change in FA/FA_0 and consequently in FA/FI .

Increasing λ_{lu}/bl increases VA^{eff} and consequently decreases FA_0 . This places a limit on the amount by which FA can change. In addition, increasing λ_{lu}/bl also increases the time constant of the exponential decrease in FA (equation (1)), thereby slowing the decrease in FA . The largest change in FA/FI is seen with small values of λ_{lu}/bl .

The interplay of these various effects was shown in figure 4, which illustrated the effect of a 25% increase in cardiac output. The gradient of the curves for the more soluble agents such as ether and methoxyflurane decrease as cardiac output increases.

The step change in cardiac output from 1 to 10 litre min^{-1} was chosen to look at the effects of solubilities over a useful range of cardiac outputs. Many applications of cardiac output measurement are at extreme values, either in patients with presumed small cardiac outputs, or in exercise physiology. Although ether, with a large λ_{bl}/g and a small λ_{lu}/bl , produced the greatest differences in FA/FI when the initial cardiac output was small, this advantage was lost when the initial cardiac output or the change in cardiac output was large. Figure 4 demonstrated that the changes seen with enflurane were approximately linear for any cardiac output change in the range 1–10 litre min^{-1} , but this was not the case for ether.

To determine if the pattern of results would have been significantly different if another cardiac output change had been studied, the optimum λ_{bl}/g for $\lambda_{lu}/bl = 1$ and a 25% increase in cardiac output (from 5 litre min^{-1} to 6.25 litre min^{-1}) was calculated in the three-breath model. This value of 3.6 was only slightly less than the optimum value for a cardiac output change from 1 to 10 litre min^{-1} of 4.1. The difference in FA/FI values for any agent with $\lambda_{bl}/g = 3.6$ compared with 4.1 was less than 4% for any of the 25% cardiac output changes studied and 4.4% for the change from 1 to 10 litre min^{-1} . These results suggest that the general pattern of results is similar over a wide range of cardiac output changes. If an

agent was required for a specific application or group of patients (for example those with decreased cardiac output states), specific calculations to determine the ideal theoretical and actual agent may be appropriate.

It would appear from these studies that the ideal soluble agent for measurement of cardiac output would be one with a moderate blood/gas solubility and a very low lung tissue/blood solubility. Such an agent would be relatively water soluble and relatively poorly soluble in fat (tissue). It would still need to diffuse readily across the alveolar membrane, so that uptake remained perfusion- rather than diffusion-limited. Of agents readily available to the anaesthetist, enflurane comes closest to this ideal.

Other factors may need to be considered when choosing a suitable agent, including the ability to increase F_I (especially for nitrous oxide) and the accuracy of the measuring system. The actual inspired concentrations of anaesthetic agents able to be used depend on the speed of onset of side effects, including sedation, and possible direct effects on cardiac output.

Enflurane may be able to replace less soluble agents such as acetylene or nitrous oxide as the soluble agent in currently used methods of cardiac output estimation by soluble uptake. Use of enflurane in these methods may produce more accurate results, and be slightly less unpleasant for the subject than agents currently used. The possible role of the volatile anaesthetic agents deserves further experimental investigation.

APPENDIX

ABBREVIATIONS AND STANDARD VALUES USED. (STANDARD VALUES SHOWN IN PARENTHESES)

$\lambda_{bl/g}$	Blood/gas partition coefficient
$\lambda_{lu/bl}$	Lung tissue/blood partition coefficient
$\lambda_{lu/g}$	Lung tissue/gas partition coefficient (= $\lambda_{bl/g} \cdot \lambda_{lu/bl}$)
V_T	Tidal volume (1 litre)
V_D	Deadspace (0.15 litre)
V_{lu}	Volume of lung tissue (0.5 litre)
V_A	Alveolar gas volume (i.e. functional residual capacity - V_D) (2.5 litre)
V_{A_0}	Alveolar gas volume at time 0 ($V_A + V_T$)
V_A^{eff}	Effective alveolar gas space, allowing for uptake by lung tissue (= $V_{A_0} + \lambda_{lu/g} \cdot V_{lu}$)
V_{bag}	Rebreathing bag volume (2 litre)
t	Elapsed time since start of breath-hold (s)
t_{in}	Inspiratory hold time (15 s for single breath; 2.5 s for three-breath model; 1 s for rebreathing)
t_{out}	Expiratory hold time (= t_{in})
F_I	Inspired concentration
F_A	Fractional concentration in alveoli after time t
F_{A_0}	Fractional concentration in alveoli at time 0
F_E	End-expired concentration
F_A^E	Fractional concentration in alveoli at end of inspiratory hold (start of expiratory hold)

F_A^E	Fractional concentration in alveoli at end of expiratory hold
F_A^I	Fractional concentration in alveoli at end-inspiration (start of inspiratory hold)
$\dot{Q}$	Pulmonary blood flow (5 litre min ⁻¹)

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ANALYSIS OF UNCERTAINTY IN THEORETICAL METHODS OF CARDIAC OUTPUT MEASUREMENT USING THE "MONTE CARLO" TECHNIQUE

R. R. KENNEDY AND A. B. BAKER

SUMMARY

We have developed three models which describe the relationship between cardiac output and the uptake of volatile anaesthetic agents, based on the Fick equation, and determined if these models could provide useful methods of cardiac output measurement. Because many variables are involved in the calculation of cardiac output using these methods, a "Monte Carlo" simulation was performed to investigate the combined effect of uncertainties in several variables on the resultant cardiac output estimate. We found that the single-breath model was most accurate when the inspired concentration was large, while the rebreathing model was better with smaller inspired concentrations. The three-breath model was the least accurate under all conditions studied. Volatile anaesthetics were generally more accurate than nitrous oxide, with both enflurane and halothane more accurate than isoflurane. The "Monte Carlo" technique provides a valuable tool for analysis of errors in measurement methods. (Br. J. Anaesth. 1993; 71: 403-409)

KEY WORDS

Heart: cardiac output. Measurement techniques: cardiac output.

Although many techniques have been described for measuring cardiac output using the rate of uptake of soluble gas [1], most are not suitable for use during routine anaesthesia. Some require specialized and bulky equipment such as volume [2-4], pressure [5] or capacitive [6] plethysmographs. Insoluble tracer gases, such as helium [7] or argon [8], may be needed to provide an estimate of lung volume, and although a rapid infra-red analyser may be used for the soluble gas, analysis of the insoluble gas usually requires use of mass spectrometry or gas chromatography [9].

When rebreathing techniques are used, the large tidal volumes (1-3 litre) and rapid ventilatory frequencies (up to 30 b.p.m.) needed to achieve equilibrium before recirculation occurs [10] may produce effects on the haemodynamic state [11] which may be especially significant in the anaesthetized patient [12]. Multiple single-breath techniques [7] combine the results of several measure-

ments made over a period of 20-30 min and are obviously unsatisfactory when conditions are unstable or changing rapidly, such as may occur during surgery and anaesthesia. An ideal method of estimation of cardiac output would be simple to perform, non-invasive, require minimal extra equipment and be suitable for use during routine anaesthesia.

We have described [13] three models based on the Fick equation which relate cardiac output to the expired concentration of a soluble gas. These models differ only in the ventilatory manoeuvre used and involve a single, long (15-s) breath-hold, three breaths at a constant inspired concentration or seven rapid breaths from a rebreathing system. We postulated that volatile anaesthetics used in our three-breath model might provide a simple means of estimation of cardiac output and require little more than a volatile anaesthetic agent and an agent analyser, both of which are often available during routine anaesthesia. We expected the three-breath method to be most useful because this model delivers the largest mass of test agent to the lungs, produces the largest expired concentrations and is the most sensitive to changes in cardiac output [13].

Two factors may limit the accuracy of cardiac output estimations using these methods. First, the change in expired concentration is not large compared with that in cardiac output. Second, a large number of variables are included in the model, not all of which can be measured or estimated easily or accurately. We were particularly interested in the combined effect of the uncertainty in several of these variables on the final estimation of cardiac output.

METHODS

A "Monte Carlo" simulation was used to study the combined effects of these errors. The technique

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allows the error resulting from the interplay of multiple errors to be estimated and confidence limits of population parameters to be calculated [14]. These results are often difficult or impossible to derive directly. After the distribution of each input parameter for the model has been derived, random values of each parameter, fitting the distribution, are found. The set of values are then combined, using the model, to produce a single result. This process is repeated a large number of times (typically several thousand) and the results collected. The distribution of the final output can then be characterized. This technique has become more practical as increased computing power has become available and has been used to analyse cardiac output estimates using both oxygen [15] and acetylene [16].

Several small programs were written in Pascal and "C" and implemented on Commodore Amiga and Apple Macintosh computers.

Standard conditions, values and abbreviations used in the simulations are listed in table I and the Appendix of the accompanying paper [13] (see p. 402). Variables were changed from these only as indicated below. All random numbers used were generated using the Macintosh Toolbox "random" function [17] and transformed to a Gaussian distribution using the procedure outlined in the Appendix of this paper.

To test the effect of multiple errors, we wrote programs which, given a "desired" mean and SD for λ_{bl}/g , λ_{lu}/bl , cardiac output, V_A , V_D , V_T , V_{lu} and F_I , calculated the mean and SD of the resulting end procedure expired concentration (FE') using a Monte Carlo technique with 2000 iterations. No error was added to t_{in} , t_{out} or V_{bag} . The same value for the other parameters of the model were used for each breath throughout any one iteration of the three-breath or rebreathing models. Other programs converted the expired concentration to equivalent cardiac output using simple linear interpolation. Values obtained were then checked by using the results in the initial model.

"Input" SD are expressed as percentage of the mean value. For example, if the stated SD is 5% then, if mean $V_A = 2.5$ litre, the actual SD of V_A is 0.125 litre, while if $V_D = 0.15$ litre, then V_D SD = 7.5 ml.

We studied halothane, enflurane, isoflurane and nitrous oxide. Nitrous oxide was included because it has been used in similar techniques for many years and because, despite recent changes in anaesthetic practice, it still has an important place in the armamentarium of many anaesthetists.

Using a Monte Carlo simulation of 2000 cycles and with cardiac output fixed (SD = 0), the mean and SD of end procedure FA was calculated for the following agents and conditions:

(1) One breath of nitrous oxide, halothane, enflurane or isoflurane with all "inputs" given SD 5%, cardiac output 5 litre min^{-1} and the duration of breath set at 2.5, 5, 7.5, 10 and 15 s, to determine the effect of duration of a single breath-hold and the choice of agent.

(2) One 15-s breath of nitrous oxide, halothane, enflurane or isoflurane with all "inputs" given SD 0.5, 1, 5 or 10%, cardiac output 5 litre min^{-1} , to

TABLE I. Reported variability in measurement of physiological parameters of the model. Values used in this paper are 1SD expressed as % of the mean

Parameter	Value used	Comments
V_A	10	Typically SD = 10–20% [18] 4% on the same day [19]
V_{lu}	18	[8]
λ	10	95% confidence limits = 20–30% [20, 21]
V_D	10	SD = 10–23%, depending on method used and initial lung volume [22]

study the effect of input error and choice of agent on the single-breath model.

(3) Three breaths or rebreathing of enflurane with all "inputs" given SD 0.5, 1, 5 or 10% and cardiac output 5 litre min^{-1} , to study the effect of input error on these models.

(4) One 15-s breath of enflurane with input SD 5% for all "inputs". Cardiac output was set at values between 1 and 10 litre min^{-1} to compare the size of "error" to the cardiac output.

(5) One 15-s breath of enflurane with input SD for solubilities and lung volumes as shown in table I, cardiac output 5 litre min^{-1} , and values for SD of F_I and V_T of 0 or 10%.

(6) One 15-s breath of enflurane with input SD for solubilities and lung volumes 50% of those in table I, cardiac output 5 litre min^{-1} and values for SD of F_I and $V_T = 0\%$.

(7) Results of the models with no input uncertainty were calculated for enflurane and nitrous oxide in the single-breath, three-breath and rebreathing models with F_I 0.5, 1.0, 1.5, 2.0 or 2.5% for enflurane and 20, 40, 60 or 80% for nitrous oxide using the values for other parameters listed in the Appendix of the accompanying paper [13] (p. 402). A normally distributed "measurement error" (mean of 0 and SD = 0.01% for enflurane and 0.1% for nitrous oxide) was added to the results of the models to study the effect of measurement errors alone. (Manuals for the Drager Irina and Datex Ultima anaesthetic agent analysers quote an accuracy of ± 0.02 vol% for volatile agents and ± 0.2 vol% for nitrous oxide.)

(8) The combined effects of uncertainty within the model and measurement errors were studied for enflurane and nitrous oxide with all three models using input values as in (6). The range of values for F_I and the measurement errors for F_I and FE' used were as in (7). The error in FE' was calculated using a second Monte Carlo process to add the measurement uncertainty to the value for FE' obtained as in examples (1)–(6) above.

Results of the Monte Carlo simulations are expressed either as the cardiac output range represented by the 2SD limits of the output concentration or as the mean of the 2SD limits expressed as a percentage of the actual value. For example, if the end procedure FA of the Monte Carlo simulation for a particular agent was a mean of 0.5 with SD 0.1, the cardiac outputs quoted are those which would produce an expired concentration of 0.3 ($0.5 - 2 \times 0.1$) and 0.7 ($0.5 + 2 \times 0.1$) using the same

model and conditions as in the Monte Carlo simulation, but without consideration of the errors. If this range of expired concentrations produced a 2SD cardiac output range of 3.5–6.4 litre min⁻¹ around an actual value of 5 litre min⁻¹, this was expressed as an 'error' of

$$[(5-3.5)/5 + (6.6-5)/5]/2 \times 100 = \pm 31\%$$

Simulations (1)–(6) above set $F_I = 1\%$ with no consideration of measurement errors. The resulting value for FE' is therefore equivalent to FA/F_I for the given proportional uncertainty in F_I .

RESULTS

As expected, increasing the duration of a single breath-hold (fig. 1) and decreasing the size of the errors (table II, fig. 2) improved the accuracy of the results; the relative "accuracy" of the agents studied

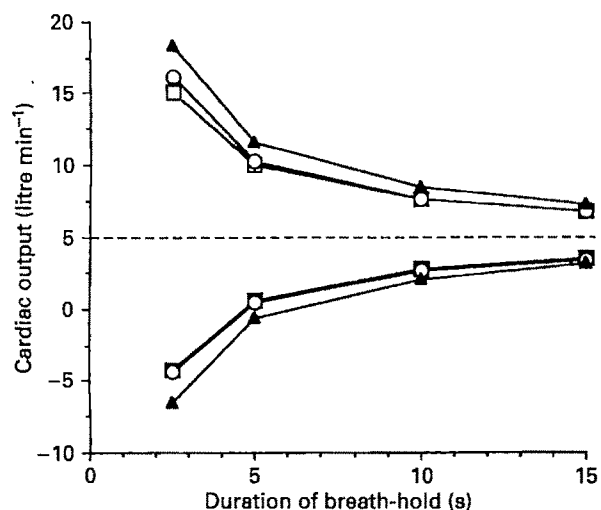


FIG. 1. Monte Carlo results for the single-breath model with isoflurane (▲), enflurane (□) and halothane (○): effects of duration of the breath-hold on the 2SD confidence limits for an actual cardiac output of 5 litre min⁻¹. All "inputs" to the model were given SD 5% of the input value, except cardiac output which was fixed at 5 litre min⁻¹ and SD = 0%.

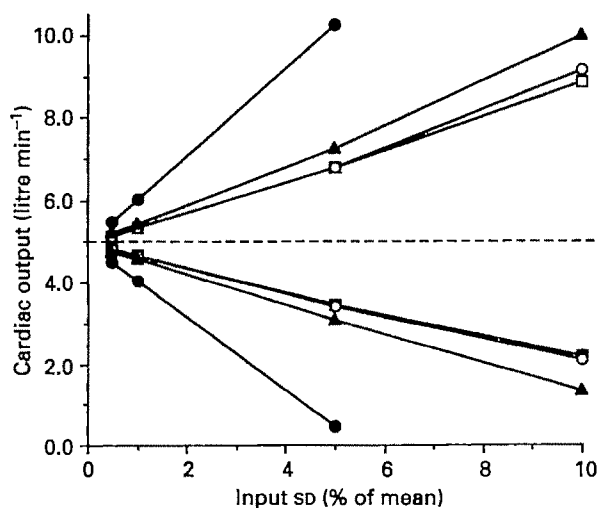


FIG. 2. Monte Carlo results for the single-breath model (15 s duration) with isoflurane (▲), enflurane (□), halothane (○) and nitrous oxide (●) showing the effect of the "input" SD on the 2SD confidence limits for cardiac output estimation. The same "input" SD was used for all variables in the model.

TABLE II. Results of Monte Carlo simulation for enflurane, showing the effects of global changes in the "input errors" on the 2SD limits of cardiac output estimation expressed as a range of cardiac outputs and as the percent deviation of the 2SD limits from the actual value of 5 litre min⁻¹

Input SD (%)	Single-breath		Three-breath		Rebreathing	
	Range	(%)	Range	(%)	Range	(%)
0.5	4.85–5.16	3.1	4.69–5.32	6.3	4.82–5.18	3.6
1	4.68–5.34	6.6	4.37–5.66	13	4.64–5.36	7.2
5	3.47–6.78	33	2.14–8.50	64	3.33–6.87	35
10	2.21–8.84	66	–0.3–13.2	135	1.74–9.24	75

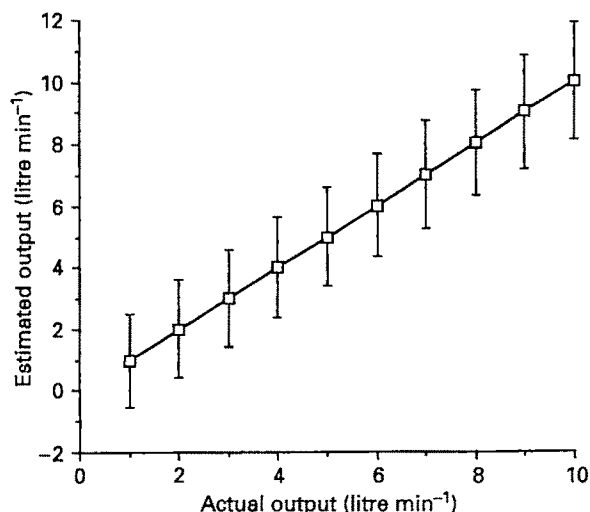


FIG. 3. Monte Carlo results for enflurane in the single-breath model for a range of actual cardiac outputs (1–10 litre min⁻¹), showing the mean and 2SD limits of the resultant cardiac output estimate. All other "inputs" were constant, with SD 5%. The calculated mean values of cardiac output estimations fall on the line of identity.

is shown in figures 1 and 2. Enflurane produced the least error (2SD limits of 33% of an actual value of 5 litre min⁻¹ for 5% input errors in the single-breath model). This result was only slightly better than that for halothane (34%) which performed much "better" than isoflurane (47%). Nitrous oxide produced a much greater "error" (166%) than any of the volatile anaesthetic agents.

Table II shows that, for enflurane, despite a predicted greater sensitivity to cardiac output changes, the three-breath technique gave far less accurate estimates of cardiac output than a 15-s single breath. Results with the rebreathing model were only slightly worse than those with the single-breath model. Similar patterns of results were seen for all agents studied. As enflurane produced the least uncertainty in all models, it was used to evaluate the effect of different combinations of errors.

When cardiac output ($\dot{Q}$) was changed while input errors were held constant at 5%, using enflurane in the single-breath model (fig. 3), the magnitude of the resultant uncertainty increased only slightly, from ± 1.5 litre min⁻¹ at $\dot{Q} = 1$ litre min⁻¹ to ± 1.91 litre min⁻¹ at $\dot{Q} = 10$ litre min⁻¹. Thus the error varied from $\pm 75\%$ at 2 litre min⁻¹ to $\pm 19\%$ at 10 litre min⁻¹ and was $\pm 33\%$ at 5 litre min⁻¹.

Using "realistic" errors from the literature with enflurane and the single-breath model, the 2SD error

TABLE III. Effect at different inspired concentrations of error in measurement of $F_{E'}$ on the uncertainty of cardiac output for nitrous oxide and enflurane, assuming no error in "input" parameters. SD of measurement error was ± 0.1 vol% for nitrous oxide and ± 0.01 vol% for enflurane. Results are the ± 2 SD limits expressed as % of the actual cardiac output of 5 litre min^{-1}

F_I	Error (%)		
	Single-breath	Three-breath	Rebreathing
Enflurane			
0.5	77	51	52
1	37	25	26
1.5	25	17	17
2	18	13	13
2.5	15	10	10
Nitrous oxide			
20	33	27	27
40	16	13	14
60	10	8.8	8.9
80	8.1	6.7	6.7

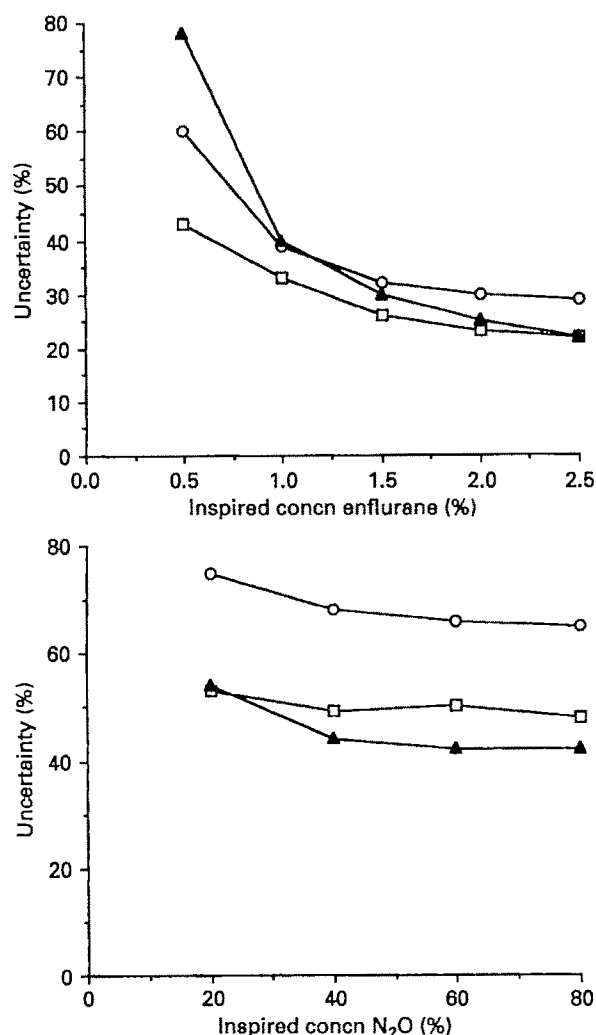


FIG. 4. Results of combining uncertainty in physiological variables with measurement error. Uncertainty is 50% of values in table I for physiological parameters and 0% for V_T . Measurement errors set at SD = 0.01% for enflurane (upper panel) and SD = 0.1% for nitrous oxide (lower panel). Results shown are percent uncertainty represented by the 2SD limits of the calculated cardiac output for an actual cardiac output of 5 litre min^{-1} . $\blacktriangle$ = Single-breath model; $\circ$ = three-breath model; $\square$ = rebreathing model.

was 31% for a cardiac output of 5 litre min^{-1} . This increased to 55% when a 10% uncertainty in V_T was added. When the "realistic" errors in physiological variables were reduced by 50% with no error in V_T , the resultant uncertainty was 15%.

Table III shows the effect, at various values of F_I , of errors in the measurement of $F_{E'}$ on calculated cardiac output for nitrous oxide and enflurane in all three models. There was little difference between the rebreathing and three-breath models, while the single-breath model produced values 20–50% worse. The error with 40% nitrous oxide was close to that with 2% enflurane.

When the measurement uncertainty was combined with the results of a Monte Carlo simulation (fig. 4), the rebreathing model was consistently better than the three-breath model, while the uncertainty in the single-breath model decreased more than in the other models as F_I increased. There was little gain in accuracy as F_I increased from 1.5% to 2.5% for enflurane or greater than 40% for nitrous oxide.

DISCUSSION

The uncertainty in the estimation of cardiac output using these models has been assessed in three ways. First, the effect of different levels of input errors was studied. This approach considered the behaviour of the system with different patterns of error, but ignored the effect of errors in the final measurement of $F_{E'}$. This is comparable to treating the system as a black box with a single output which can be measured exactly, and is useful for examining the interplay of potential errors. Second, possible actual levels of error in the estimation of various variables were considered using values from the literature. It is difficult to distinguish between variability occurring as a result of errors in measurement and those caused by changes in the actual values. Finally, the combined effects of varying inspired concentration, errors from the physiological "black box" and measurement errors were studied in an attempt to approximate the actual application of these models.

Decreasing the magnitude of "input" errors or increasing the duration of the single breath decreased the resultant uncertainty. Increasing the duration of the manoeuvre decreased uncertainty because the amount of gas taken up increased almost linearly with increasing time. Thus, if the time available for uptake doubled, the difference between final concentrations at different cardiac outputs was nearly double that at the shorter time interval.

We were surprised initially that the three-breath model performed so poorly, despite being much more "sensitive" to cardiac output changes than the single-breath model, as shown in table II of the accompanying paper [13] (p. 401). One possible explanation for this result is the effect of a number of the variables being re-used for each breath; for example, a value for tidal volume is used five times in the three-breath model. The total number of variables used in calculating the final result, and hence the compound effect on uncertainty, is greater than in the single-breath model. This argument suggests that the rebreathing model should be even less

accurate than the three-breath model, as seven breaths are taken with a consequent increase in the number of variables used. In fact the rebreathing model was almost as accurate as the single-breath model (table II) and consistently better than the three-breath model. The rebreathing model was much less sensitive to V_T changes than the other models. A 10% increase in V_T produced a 10.8% increase in FA/FI in the single-breath model, an 8.8% increase in the three-breath model, but a 0.2% decrease in the rebreathing model. This decreased sensitivity to gas volume changes may act to minimize the effect of re-use of many of the variables in the Monte Carlo simulations.

As shown in figure 3, the magnitude of the "error" in the cardiac output estimate remained nearly constant (± 1.5 to ± 1.9 litre min^{-1}). The uncertainty in the results of the single-breath model, expressed as a proportion of the true value, increased as cardiac output was decreased. As all the sources of error were held constant, the calculated error in expired concentration remained approximately constant. As the expired concentration-cardiac output relationship was approximately linear for all the agents studied in this paper, it follows that there should be a constant magnitude of uncertainty in the final result. A similar pattern was seen with the other models. The most frequent applications of cardiac output measurements are either in patients with small cardiac outputs, or in exercise physiology, when outputs are normal or increased. This pattern of results suggests that soluble gas uptake methods of cardiac output estimation may be more accurate in the exercise physiology laboratory than in the operating theatre.

Table I lists the variability in the measurement of several of the model parameters quoted in the literature. It is not possible to distinguish between uncertainty in the measurement methods and actual changes in the parameter being measured. Values used in this paper are at the low end of quoted ranges. Despite this, another 50% reduction in the magnitude of these uncertainties, which might approximate within-patient variability over a short time period, combined with no uncertainty in V_T or FI is needed to reduce the error to an acceptable level.

When the effect of error in the measurement of the final FE' was considered without any uncertainty in input variables and hence none in the actual value being measured (table III), the three-breath and rebreathing models produced similar results and were more accurate than the single-breath model. Values for enflurane were comparable to those for nitrous oxide, with a 20-fold increase in FI . The results in the companion paper [13] show the magnitude of the change in FA/FI for nitrous oxide was 50% that of enflurane for a given change in cardiac output. Combined with a 10-fold reduction in the accuracy of infra-red analysers (from ± 0.02 vol% for enflurane to ± 0.2 % for nitrous oxide), it would be expected that an increase of about 20 times in the inspired concentration would produce a change in FE' of the same magnitude for the two agents, as shown in table III.

The results in table III could, optimistically, be considered to be the relative errors that could be obtained with repeated measurements in the same patient and with the test gas being obtained from a single source. These results show a distinct advantage for nitrous oxide compared with enflurane. An uncertainty of less than 15% is obtained with FI 2% or more for enflurane or 40% nitrous oxide or more, using either of the multiple-breath models. This compares favourably with thermodilution methods [23]. Unfortunately, the assumption that VA , VD , Vlu and λ all remain constant is unlikely to be true, as several of the variables may change both with anaesthesia itself (VA as approximated by changes in functional residual capacity [24]) or as a result of changes in cardiac output (VD) [25].

Measurement error was combined with the effect of a small but possibly attainable level of uncertainty (fig. 4). In this situation, the rebreathing model was consistently more accurate than the three-breath mode, but the single-breath model "improved" more rapidly than the others as FI increased. Enflurane in the single-breath method was almost twice as inaccurate as the rebreathing model when FI was 0.5%, but the two models were equal when FI was 2.5%. The lack of dramatic increase in accuracy of the rebreathing model with increasing FI for nitrous oxide, and to some extent for enflurane, is in accord with the finding that acetylene concentration may be reduced from 2% to 0.3%, which is tolerated far better by subjects, without significant effects on the accuracy of the results of rebreathing methods [16].

Table II ranks the models in order of decreasing accuracy as single-breath-rebreathing-three-breath. This is also seen in figure 4 for large inspired concentrations. The probable reason for the inaccuracy of the single-breath model with small inspired concentrations is the very small expired concentrations, which amplified the effect of uncertainty in the measurement of FE' . For a single breath of enflurane under the conditions of figure 4 when $FI = 0.5\%$, $FE' = 0.055$ (SD 0.010)% and at $FI = 2.5\%$, $FE' = 0.273$ (SD 0.015)%, representing a five-fold difference in FE' but only a 50% change in SD. In contrast, in the rebreathing model, FE' increased by about the same magnitude (from 0.122 to 0.611%), while SD nearly doubled (from 0.011 to 0.021%). These values suggest that the uncertainty in the final cardiac output estimation improved much more as FI increased in the single-breath model than in the rebreathing model.

Although the most accurate of the results presented in this paper are comparable to the variability present in other established methods of cardiac output measurement [23], there are other potential sources of error which have not been considered in this analysis. First, no allowance has been made for uncertainty in the actual measurement of time intervals and in both the three-breath and rebreathing models, all inspiratory and expiratory times were assumed to be identical. Second, it has been shown that, for such manoeuvres, the time taken as the zero point can significantly alter the results [26, 27], because the exact time at which the gases

appear to reach and become mixed in the alveoli can vary up to 2 s, after the start of inspiration [27]. Third, the pattern of ventilation considered here was very stylized, with no consideration of intermediate lung volumes and concentrations. It is unlikely that use of a more complex model of ventilation and uptake, such as that of Martonen and Wilson [26], would improve the accuracy of the results significantly because it is primarily the size of the breath rather than the rate of change between inspiration and expiration which is responsible for the errors. Fourth, the effect of uneven ventilation and perfusion have not been considered. The pattern of ventilation and perfusion seen in normal man does not appear to affect significantly the accuracy of cardiac output estimations until FEV_1 is less than 60% of predicted [11, 28]. It is also unlikely that a method based on this model could be improved by use of a different agent such as acetylene, as enflurane is close to the "ideal" agent for this type of measurement, as outlined in our companion paper [13].

Mathematical and computer modelling can be powerful tools which may produce unexpected results. These techniques require precise definition of a problem and allow theoretical experiments, which may be repeated many times. However, the limitations of any model and method of analysis must be borne in mind when extrapolating results of a model. All models only approximate the system under study, and therefore experimental validation is important [29]. The various components of the model and analysis described here have been validated in several ways. Several studies [1, 4, 7, 8, 10, 12, 28, 30–32] have confirmed the utility of the basic exponential equation, while the theoretical work of Martonen and Wilson [26] suggests that the model of ventilation used here did not greatly affect the result. A comparison of theoretical and measured "errors" in cardiac output estimation using different concentrations of acetylene [16] demonstrated the utility of the Monte Carlo technique in this type of application. Even with this background, the predictions of any model should be seen only as a guide and a method of limiting the number of experiments needed to establish the validity of these predictions [33].

Several methods based on analysis of expired soluble gases have been shown to provide consistent results which parallel those obtained by thermodilution or direct Fick methods [2, 27, 28, 31, 32, 34, 35]. That these more complex methods produce repeatable estimates of cardiac output may be because they overcome the uncertainty predicted by our models by obtaining multiple measurements of the changing gas concentration during rebreathing [27, 35] or a long single expiration [28, 32], in contrast to taking a single end procedure measurement. Use of an inert tracer, such as helium or argon, provides an indication of lung gas volume, while the inclusion of carbon monoxide in the gas mixture allows the true time zero for alveolar mixing to be determined [26, 27]. However, rapid analysis of such a gas mixture, containing carbon monoxide, argon and one (or more [31]) soluble gases, requires use of

a mass spectrometer rather than a relatively simple infra-red anaesthetic agent analyser.

The aim of the work described in this paper was to use computer implementations of simple models to assess the "errors" in the estimation of cardiac output based on the uptake of volatile anaesthetic agents. The Monte Carlo technique was used to evaluate these models and is a valuable tool for this type of analysis. It has been demonstrated that the three-breath technique for measuring cardiac output, which theoretically appeared to be the most sensitive to cardiac output changes, was considerably less accurate when uncertainties in the large number of variables involved were considered. The single-breath method was most accurate when errors in the measurement of FE' were excluded or when large inspired concentrations were used. The accuracy of the rebreathing method was not greatly affected by changes in Fi . Collecting multiple data points during a single manoeuvre may increase the accuracy of all models sufficiently to produce useful measurement techniques based on the rebreathing or single-breath models. Of the agents studied, enflurane was associated with the least amount of uncertainty and was generally associated with much less error than the use of nitrous oxide, except when there was no uncertainty or variability in the physiological parameters of the model.

APPENDIX

GENERATION OF NORMAL RANDOM NUMBERS

A sample of n rectangular random numbers $a \leq x \leq b$, such as produced by a standard random number generator has a mean = $(a+b)/2$ and SD of $\sqrt{[(b-a)^2]/12 \cdot n}$. If $a = 0$ and $b = 1$, then mean = 0.5 and SD = $\sqrt{[1/12 \cdot n]}$ [36].

Therefore, if we take 100 random numbers and calculate the mean of these values, this result can be transformed to fit the desired distribution by scaling SD and then the mean appropriately. This is illustrated in the segment of Pascal code which produces a single random number drawn from a normal distribution with a mean of "mean_wish" and a standard deviation of "sd_wish".

```
function rand_norm (sd_wish, mean_wish: real): real;
var
    i: integer;
    sd: real;
const
    cycles = 100;
begin
    rand_norm := 0;
    sd := sqrt(1/(12*cycles));
    for i := 1 to cycles do
        rand_norm := rand_norm +
            abs(random/maxint);
    {random returns a value in range-maxint ≤ x < maxint, convert to
    0 ≤ x < 1}
    rand_norm := rand_norm/cycles - 0.5;
    rand_norm := rand_norm*sd_wish/sd + mean_wish;
end;
end.
```

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PHENOTYPES ASSOCIATED WITH MALIGNANT HYPERTHERMIA SUSCEPTIBILITY IN SWINE GENOTYPED AS HOMOZYGOUS OR HETEROZYGOUS FOR THE RYANODINE RECEPTOR MUTATION†

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SUMMARY

We have examined the phenotypic expression of several parameters associated with malignant hyperthermia (MH) susceptibility in three groups (homozygous normal, homozygous abnormal and heterozygous) of Yorkshire/Duroc swine genotyped by a mutation in the ryanodine receptor. Subgroups of homozygous abnormal were classified further by the appearance or absence of muscle rigidity on prolonged in vivo challenge with halothane and suxamethonium. Four swine heterozygous for the proposed MH mutation were indistinguishable from five homozygous normal swine in temperature, heart rate, lactate concentrations, base excess and pH determined during the prolonged halothane and suxamethonium challenge. Resting creatine kinase concentrations, the in vivo barnyard challenge, the in vitro contracture response of skeletal muscle to 3% halothane and the threshold for Ca^{2+} -induced Ca^{2+} release were also similar for subgroups of homozygous normals and heterozygotes. Therefore, inheritance of only one allele carrying the defect in the ryanodine receptor does not significantly alter phenotypes associated with MH susceptibility in this strain of swine. As four swine homozygous for the proposed MH defect did not exhibit rigidity and three of these had no other signs of MH on prolonged halothane and suxamethonium challenge, we conclude that the reported mutation in the ryanodine receptor may be necessary, but is not sufficient, for consistently eliciting the malignant hyperthermia syndrome. These findings suggest that a modulator of the syndrome may explain variability within individuals in human MH. (Br. J. Anaesth. 1993; 71: 410–417)

KEY WORDS

Hyperthermia: malignant, porcine, phenotypes, genotypes.

Anaesthesia-induced malignant hyperthermia (MH) is a potentially fatal inherited disorder of skeletal muscle [1–3] manifested typically by muscle rigidity, hyperthermia, hyperkalaemia, tachycardia, signs of lactic acidosis and greatly increased myoplasmic

concentrations of Ca^{2+} in the response to halothane [4].

Swine have been used as an animal model of MH, as they may exhibit an inherited MH-like syndrome in response to stress or anaesthetics [5]. In humans, the syndrome varies greatly from individual to individual and even in the same individuals at different times, such that at times an episode may not occur [6, 7]. Similar observations have not been reported in swine. However, age is an important factor for expression of porcine MH [8] and variation in the degree of response between strains of swine has been reviewed [5]. These findings suggest that one or more factors in addition to the MH gene modulate the expression of the syndrome in humans and swine.

There is evidence suggesting that the threshold of Ca^{2+} -induced Ca^{2+} release may be reduced in MH-susceptible muscle [9–11]. However, in isolated terminal cisternae preparations from MH muscle, the dose-response profile for halothane-induced Ca^{2+} release is normal [9, 11–14], suggesting that factors other than, or in addition to, a defective Ca^{2+} release channel, or ryanodine receptor, are essential for the exaggerated response of the MH sarcoplasmic reticulum to halothane. More recently, a specific polymorphism (C-to-T nucleotide substitution at position 1843) in the gene encoding the ryanodine receptor (*ryr1*), causing a Cys⁶¹⁵ for Arg⁶¹⁵ amino acid substitution, has been associated with MH susceptibility in swine [15]. Five to 10% of the swine homozygous for this mutation do not exhibit an MH episode on barnyard challenge with halothane [16, 17]. It has been argued that the barnyard challenge is less sensitive than a prolonged challenge with halothane and suxamethonium and may not detect all MH-susceptible subjects. However, as no other studies verified that the pigs would trigger with a prolonged halothane and suxamethonium challenge, this finding could also be interpreted as a variable

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expression of MH dependent on a modifying factor. Recent, highly detailed, functional studies of ryanodine receptor activity have detected defects in MH susceptible humans that are similar to those in a subpopulation of humans that are phenotypically not MH susceptible as regards their *in vivo* response to anaesthetics [18]. All of the above observations suggest that abnormal ryanodine receptor function alone is insufficient for expression of the MH syndrome.

The present study is the first to examine a wide variety of phenotypes associated with well-defined MH genotypes, including heterozygotes, in a strain of swine derived from a Yorkshire/Duroc cross. This study also examined whether the proposed MH genotype is sufficient for expression of the MH syndrome in Yorkshire/Duroc swine, or whether individuals from this strain of swine exhibit variability in the response to triggering agents, as has been reported for humans.

MATERIALS AND METHODS

Thirty tissue specimens from swine previously examined for a variety of phenotypes (table I) had been preserved in liquid nitrogen. The genomic DNA in these specimens was analysed for the identified porcine mutation, as described below. We did not have complete phenotyping for all the genotyped swine.

In vivo challenge and clinical laboratory findings

The swine (Yorkshire/Duroc cross) were obtained from Iowa State University. The study was approved by the Hahnemann University Animal Welfare Committee. From 1 to 6 weeks after their arrival at Hahnemann University, the pigs (15–40 kg) were anaesthetized with non-triggering agents (xylazine, ketamine, nitrous oxide), as described previously [19]. Biopsies of the gracilis and longissimus dorsi muscles for *in vitro* contracture testing and Ca^{2+} regulation studies, respectively, were removed under non-triggering anaesthesia immediately before the *in vivo* challenge with halothane and suxamethonium. The swine were tested for MH susceptibility by the North American procedure [20], using the *in vitro* contracture response of gracilis muscle to halothane and caffeine, as described previously in detail [19, 21]. Briefly, an *in vitro* contracture response greater than 0.7 g to halothane (3% in the gas phase) in any one of three muscle fibre bundles tested, or a contracture greater than 0.3 g to caffeine 2 mmol litre⁻¹ in any one of the three muscle fibre bundles tested, is considered a response indicative of MH susceptibility in our laboratory. After the muscle biopsies, the pigs were challenged *in vivo* with 3% halothane for 5 min, followed by 2% halothane for an additional 43 min. Twenty minutes after the halothane concentration was reduced to 2%, suxamethonium (1 mg kg⁻¹ per dose) was administered five times at 5-min intervals. Blood was obtained for analysis before the halothane administration and 3 min after the last suxamethonium administration. The serum K^+ and lactate concentrations, base excess and pH were measured by the Hahnemann

University Hospital Clinical Laboratory. Temperature was monitored by both an oesophageal probe and a probe inserted about 2 cm into the gluteal muscle. Muscle rigidity was determined subjectively by the resistance of the limbs to movement. Rigidity in all cases was accompanied by complete extension of the limbs and these could not be bent at the joints. In the absence of rigidity, in all cases the limbs were completely flaccid and could be easily moved.

Polymerase chain reaction and restriction endonuclease analysis of the C to T polymorphism at nucleotide 1843

Genomic DNA was extracted from skeletal muscle (1–2 g of longissimus dorsi) that had been stored in liquid nitrogen. The tissue was homogenized in 0.5% sarkosyl, sodium citrate 25 mmol litre⁻¹, β -mercaptoethanol 0.1 mol litre⁻¹ and guanidinium thiocyanate 4 mol litre⁻¹ and extracted subsequently with phenol:chloroform (1:1 v/v). The aqueous phase was removed and precipitated with isopropanol. The precipitate was washed with lithium chloride 4 mol litre⁻¹ and resuspended in Tris 10 mmol litre⁻¹, pH 7.5, EDTA 1 mmol litre⁻¹, SDS 0.5%. The DNA was extracted with chloroform and the aqueous phase precipitated with isopropanol. The precipitate was washed with ethanol 70%, air dried and resuspended in sterile water. Polymerase chain reaction (PCR) was conducted under the conditions described by Fujii and colleagues [15], using the primers 5'-GTTCCCTGTGTGTGTGCAATGGTG-3' and 5'-ATCTCTAGAGCCAGGGAGCAAGTTCTCAGTAAT-3' (Operon Technologies, Inc., Alameda, CA). Because the point polymorphism alters the restriction enzyme site [15], the PCR products generated from the *ryr1* gene were digested with the restriction enzymes *Hin* PI and *Hgi* AI (New England BioLabs, Beverly, MA) to confirm the presence or absence of the polymorphism for both alleles at nucleotide 1843. The PCR product was incubated with *Hin* PI 15 u or *Hgi* AI 10 u at 37 °C for 16 h. The restriction products were then electrophoresed on an 8.5% acrylamide/1.1% bis-acrylamide gel at 100 V for 2 h in TBE buffer (Tris 89 mmol litre⁻¹, boric acid 89 mmol litre⁻¹, EDTA 2 mmol litre⁻¹). The gels were stained with ethidium bromide.

Threshold of calcium-induced calcium release assay

The threshold of Ca^{2+} -induced Ca^{2+} release was determined on a heavy sarcoplasmic reticulum fraction isolated from longissimus dorsi removed under non-triggering anaesthesia, as described previously [11, 22]. In brief, an 8000–12000 g fraction was isolated by differential centrifugation [10] and the threshold of Ca^{2+} -induced Ca^{2+} release was determined in the presence of pyrophosphate [23] to increase the sensitivity of the assay. Calcium was added in 10- μ mol litre⁻¹ pulses and the uptake and eventually the release of Ca^{2+} from the vesicles monitored with arsenazo III [10, 11, 22]. (We have recently identified a systematic error in our previously published values for the threshold of Ca^{2+} -induced Ca^{2+} release. To compare the present study with previous studies from our laboratory, the values

TABLE I. Phenotypes examined for each swine genotyped for the proposed porcine MH mutation. The groups were subdivided by genotype, including homozygous normal (C/C), heterozygotes (C/T) and MH susceptibles (T/T). n.d. = Not determined. * Reactions of red blood cells with antisera for the H system (Ha or Hc), or with either the A or O blood factors. — = no reaction [24]. † Either muscle rigidity (+) or no rigidity (–) within 3 min of an in vivo challenge with 6% halothane. ‡ Outcome either MH susceptible (+), or normal (–). Y = Details recorded; N = details not recorded for the prolonged in vivo halothane and suxamethonium challenge; NR = no rigidity; R = muscle rigidity during the challenge. Threshold = threshold of Ca²⁺-induced Ca²⁺ release

Pig No.	Blood type*		Resting CK value (u litre ⁻¹)	Barnyard challenge†	Contracture test‡	In vivo challenge	Threshold (μmol Ca ²⁺ /mg protein)
	H-type	A-O					
Homozygous C/C (normal)							
1	c/c	O	39	—	—	Y/NR	1.7
2	c/c	A	18	—	—	Y/NR	4.1
3	c/c	A	13	—	—	Y/NR	6.1
4	c/c	O	12	—	—	Y/NR	4.2
5	c/c	A	12	—	—	Y/NR	4.0
6	a/c	O	27	—	—	N/NR	n.d.
7	n.d.	n.d.	n.d.	—	n.d.	N/NR	2.6
Heterozygotes (C/T)							
8	c/c	O	91	—	—	Y/NR	2.0
9	c/c	O	58	—	—	Y/NR	2.6
10	c/c	O	14	—	—	Y/NR	4.2
11	c/c	O	27	—	—	Y/NR	n.d.
12	a/a	O	36	—	—	N/NR	n.d.
13	a/c	O	36	—	—	N/NR	n.d.
14	a/a	O	32	—	—	N/NR	n.d.
15	a/c	O	46	—	—	N/NR	n.d.
Homozygous T/T (MH susceptible)							
16	a/a	—	270	+	+	Y/R	1.7
17	a/a	—	301	+	+	Y/R	2.8
18	a/-	O	130	+	+	Y/R	3.8
19	a/a	n.d.	927	+	+	Y/R	1.9
20	a/a	n.d.	128	+	+	Y/R	1.6
21	a/a	—	394	+	+	Y/R	4.1
22	a/a	—	492	+	+	Y/NR	1.6
23	a/-	O	1140	+	+	Y/NR	3.0
24	a/-	O	153	+	+	Y/NR	2.7
25	a/a	O	211	+	+	Y/NR	1.4
26	a/-	O	316	+	+	N/R	n.d.
27	a/-	O	157	+	+	N/R	n.d.
28	a/a	—	272	+	+	N/R	1.7
29	a/a	—	499	+	+	N/R	2.1
30	a/-	O	216	+	+	N/R	n.d.

in the older studies (accepted before January, 1993) must be divided by a factor of two.)

RESULTS

Distribution of phenotypes examined for each genotype

A total of 30 swine were genotyped and the phenotypes examined for each of these are identified individually in table I. Of the 30 swine, 15 were diagnosed initially as MH susceptible by barnyard challenge (rigidity to 6% halothane within 3 min), A–O and H blood typing and creatine kinase (CK) values (table I) and these were all found to be homozygous for the proposed MH mutation. An additional 15 swine were diagnosed initially as not being MH susceptible by the same criteria and, of these, eight were heterozygous for the proposed MH mutation and seven were homozygous normal. The 15 MH-positive swine and 14 of the 15 MH-negative swine were diagnosed for MH susceptibility by the halothane and caffeine contracture tests also, using the North American procedure. All the swine were challenged *in vivo* with halothane and suxamethonium, but detailed clinical data (temperature, heart rate, lactate concentrations, base excess, pH)

were obtained for only five of the seven homozygous normals, four of the eight heterozygotes and 10 of the 15 homozygous MH-positive swine. The 10 homozygous MH-positive swine in which clinical data were gathered were challenged with halothane and suxamethonium and were grouped further into those exhibiting rigidity (*n* = 6) and those in which no rigidity was observed (*n* = 4), in order to examine if other physiological and biochemical changes associated with MH were observed in the absence of rigidity during the halothane and suxamethonium challenge.

In vivo challenge with halothane and suxamethonium

Two of the rigid group of MH-susceptible swine exhibited rigidity to halothane alone and all five exhibited rigidity after the first administration of suxamethonium. The non-rigid MH susceptibles had no signs of rigidity even after five bolus injections of suxamethonium.

Starting temperature has been suggested to influence the response of MH-susceptible swine to triggering anaesthesia [25]. The oesophageal temperatures before administration of halothane (mean (SEM)) for the homozygote controls (36.6 (0.1) °C), heterozygotes (38.1 (1.6) °C), homozygote MH sus-

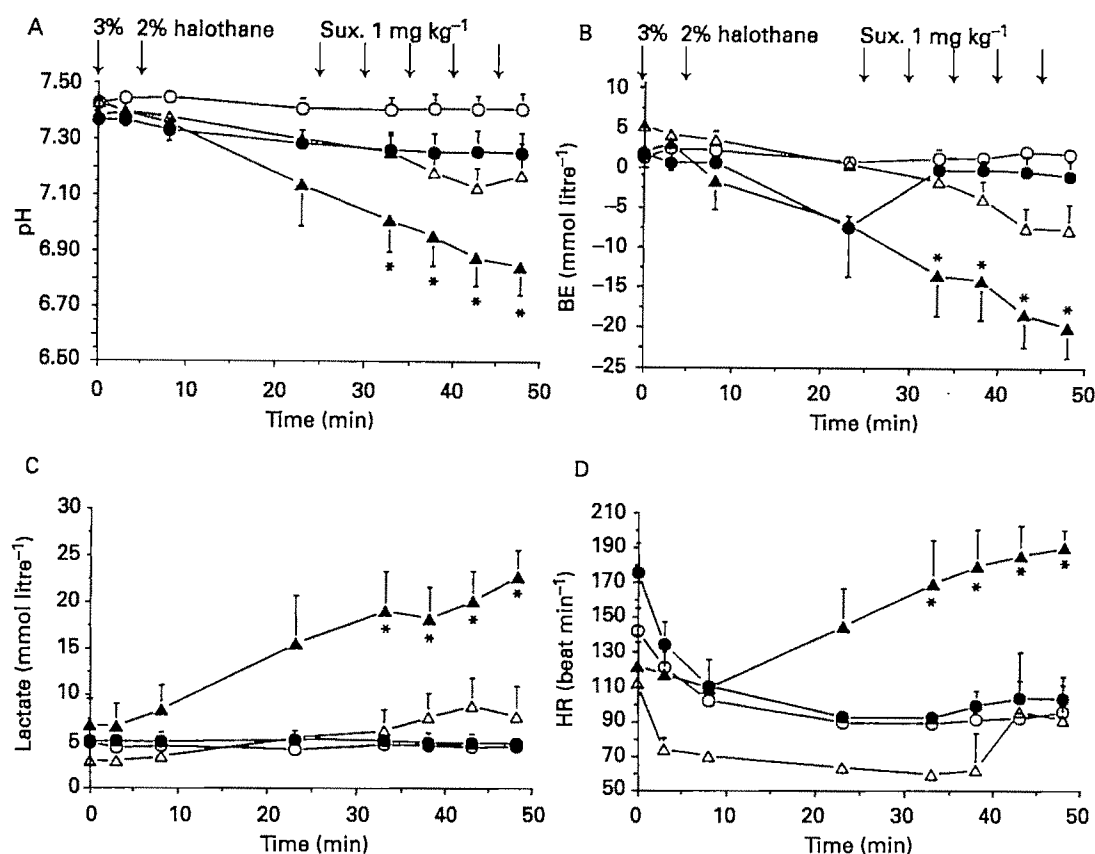


FIG. 1. Mean (SEM) changes in (A) pH; (B) base excess (BE); (C) lactate concentration; and (D) heart rate (HR) accompanying prolonged *in vivo* challenge with halothane and suxamethonium. Blood was obtained at time 0, which was immediately before addition of 3% halothane for values in A–C. Heart rate was also recorded immediately before addition of halothane for time 0. Halothane was reduced to 2% as indicated by the arrow and maintained at 2% for the remainder of the challenge. Suxamethonium (Sux.) 1 mg kg⁻¹ was injected five times, as indicated. Genotypes: -/- = homozygous normal (C/C) (○) (n = 5); -/+ = heterozygous (C/T) (●) (n = 4); +/+(+) = homozygous MH susceptible (T/T) exhibiting rigidity on prolonged halothane and suxamethonium challenge (▲) (n = 6); +/-(-) = homozygous MH susceptible (T/T) not exhibiting rigidity on prolonged halothane and suxamethonium challenge (△) (n = 4). The four groups were tested at each time point by a one-way ANOVA and Scheffé test.

* Significantly different from control ($P < 0.05$).

ceptibles exhibiting rigidity (36.4 (0.6) °C) and homozygote MH susceptibles not exhibiting rigidity (37.1 (1.1) °C) were not significantly different (one-way ANOVA; $P > 0.05$). Only the oesophageal temperature for the homozygote MH susceptibles exhibiting rigidity was increased significantly ($P < 0.05$; two-tailed paired t test) at the end of the halothane and suxamethonium challenge. The final oesophageal temperatures were 36.2 (0.1) °C, 37.8 (1.7) °C, 37.6 (0.6) °C and 37.4 (1.1) °C for the four groups, respectively. Muscle temperatures (gluteal) for the homozygote controls (35.4 (0.6) °C), heterozygotes (37.3 (0.3) °C), homozygote MH susceptibles exhibiting rigidity (36.9 (0.7) °C) and homozygote MH susceptibles not exhibiting rigidity (35.4 (0.9) °C) were also not significantly different (one-way ANOVA; $P > 0.05$) before the halothane challenge. The muscle temperature for the homozygote MH susceptibles exhibiting rigidity (37.7 (0.4) °C) was not significantly increased ($P = 0.07$; two-tailed paired t test) at the end of the halothane and suxamethonium challenge, nor was muscle temperature increased for the other groups (data not shown).

During the prolonged halothane and suxamethonium challenges, the serum pH (fig. 1A)

and base excess (fig. 1B) decreased significantly only for the MH-susceptible group exhibiting rigidity. The pH values for two of the four heterozygotes (7.46, 7.28, 7.13, 7.12) and two of the four homozygote MH susceptibles not exhibiting rigidity (7.41, 7.29, 7.16, 6.88) were within the range for homozygous normals (7.21–7.52) after 48 min. The base excess values for all of the homozygous normals (range +0.4 to +4.2) and two of the four heterozygotes (+0.2, +3.4, -1, -6 mmol litre⁻¹) were positive after 48 min. Base excess values were negative—both for the MH susceptibles not exhibiting rigidity (-2, -3.7, -8 and -16.5 mmol litre⁻¹) and for the MH susceptibles exhibiting rigidity (-6, -17, -22, -30, -30 mmol litre⁻¹). Serum lactate concentrations (fig. 1C) and heart rate (fig. 1D) increased significantly only for the MH-susceptible group exhibiting rigidity. The lactate values for three of the four heterozygotes (2.7, 4.2, 6.2, 6.9 mmol litre⁻¹) and two of the four homozygote MH susceptibles not exhibiting rigidity (1.3, 5.7, 7.6, 17 mmol litre⁻¹) were less than the greatest values in homozygous normals (range 2.6–6.5 mmol litre⁻¹) after 48 min. All these variables became significantly different from control only after the second addition of suxamethonium. Serum K⁺

concentrations at the end of the challenge were not significantly different between the groups, as determined by one-way ANOVA (data not shown). The difference in muscle tone between rigid and non-rigid pigs was unmistakable. The heart rate, lactate concentrations and pH values in all but one case supported the subjective determination of rigidity for the MH swine. The lactate value ($17 \text{ mmol litre}^{-1}$) for one non-rigid MH pig was greater than that of one of the rigid MH pigs ($16 \text{ mmol litre}^{-1}$) and the pH value (6.88) was smaller only than that in the same rigid MH pig (7.29). While slight increases in muscle tone may have occurred in response to this extreme *in vivo* challenge in the four non-rigid MH swine, these would not be detected or interpreted as clinical signs of MH by an anaesthetist.

In vitro contracture response studies

Other investigators have reported that several strains of swine heterozygous for the MH defect have intermediate [26], or MH-like [27] *in vitro* contracture responses to halothane. However, neither of these studies directly genotyped the heterozygotes. Therefore, we determined the phenotypic expression of the heterozygotes in pigs genotyped for the mutation in the ryanodine receptor in the Yorkshire/Duroc strain. First, all the homozygous normal and heterozygote swine tested were diagnosed as normal by the North American MH Group procedure (table I). All homozygous MH-susceptible swine were diagnosed as MH susceptible (table I). Based on the maximum response for the three strips tested, the *in vitro* contracture response to 3% halothane in homozygous controls ($0.07 (0.03) \text{ g}$; $n = 6$) was not significantly different (two-tailed grouped *t* test) from that of the heterozygotes ($0.28 (0.09) \text{ g}$; $n = 8$) and there was considerable overlap between the groups. These values for controls and heterozygotes are similar to the human control values reported by the North American MH Group [28]. The MH susceptibles had markedly stronger responses to halothane ($1.42 (0.13) \text{ g}$; $n = 15$) than either the heterozygotes or homozygous normals.

Resting CK values

The resting CK values were significantly different ($P < 0.05$): $20 (4) \text{ u litre}^{-1}$ ($n = 6$) for the homozygous controls and $43 (8) \text{ u litre}^{-1}$ ($n = 8$) for the heterozygotes. However, there was considerable overlap in values, making it impossible to differentiate between homozygous normals and heterozygotes by CK values alone (table I). The CK value for MH susceptibles ($374 (76) \text{ u litre}^{-1}$; $n = 15$) were considerably greater than those of the other groups in all cases (table I).

Threshold of Ca^{2+} -induced Ca^{2+} release in a heavy sarcoplasmic reticulum fraction

The threshold of Ca^{2+} -induced Ca^{2+} release has been reported to be reduced in heavy sarcoplasmic reticulum fractions from MH-susceptible skeletal muscle [9–11]. The observation was supported in the present study for preparations from MH swine,

TABLE II. Threshold of Ca^{2+} -induced Ca^{2+} release in genotyped swine (mean (SEM)). Genotypes: $-/-$ = homozygous normal (C/C); $-/+$ = heterozygous (C/T); $+/+(+)$ = homozygous MH susceptibles (T/T) exhibiting rigidity on prolonged halothane and suxamethonium challenge; $+/+(-)$ = homozygous MH susceptibles (T/T) not exhibiting rigidity on prolonged halothane and suxamethonium challenge. * Significantly less than $-/-$ ($P < 0.05$; one-tailed *t* test)

Genotype	<i>n</i>	Threshold ($\mu\text{mol Ca}^{2+}/\text{mg protein}$)
$-/-$	6	3.8 (0.6)
$-/+$	3	3.1 (0.5)
$+/+(+)$	8	2.5 (0.4)*
$+/+(-)$	4	2.2 (0.4)*

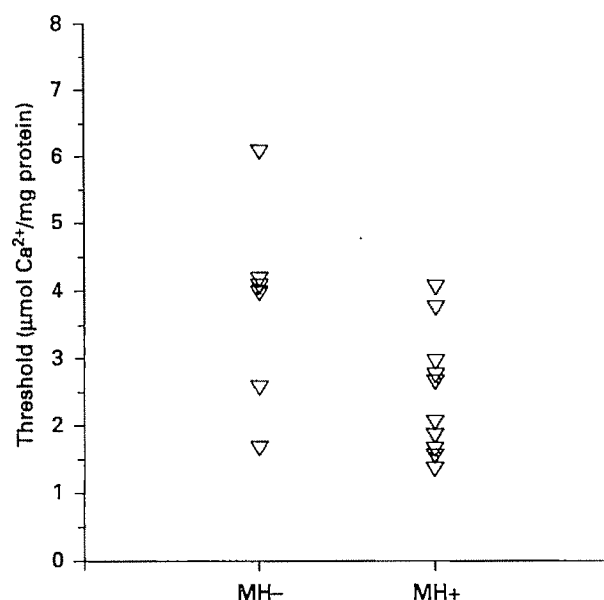


FIG. 2. The threshold for Ca^{2+} -induced Ca^{2+} release in swine diagnosed as normal (MH-) (homozygous normals and heterozygotes: mean $3.6 (SEM) 0.4 \mu\text{mol Ca}^{2+}/\text{mg protein}$; $n = 9$) or susceptible to MH (MH+) (rigid plus non-rigid: mean $2.4 (0.3) \mu\text{mol Ca}^{2+}/\text{mg protein}$; $n = 12$) by the *in vitro* contracture test. Three MH-negative swine had threshold values of $2.6 \mu\text{mol Ca}^{2+}/\text{mg protein}$ and two had values of $4.2 \mu\text{mol Ca}^{2+}/\text{mg protein}$. Two MH-positive swine had values of $1.7 \mu\text{mol Ca}^{2+}/\text{mg protein}$ and two had values of $1.6 \mu\text{mol Ca}^{2+}/\text{mg protein}$.

whether or not they exhibited rigidity on prolonged halothane and suxamethonium challenges (table II). The values for the three heterozygotes (table I) were not statistically different from normals ($P = 0.43$ by two-tailed *t* test) (table II). To test the association of a reduced threshold of Ca^{2+} -induced Ca^{2+} release with outcome of the diagnostic contracture test for MH, the values for the heterozygotes and homozygote normals were pooled to form the MH-negative group and the values from the homozygous MH-positive swine with and without rigidity on prolonged *in vivo* halothane and suxamethonium challenge were pooled for the MH-positive group in figure 2. Overall, the threshold of Ca^{2+} -induced Ca^{2+} release of swine diagnosed as MH-positive was significantly less than that from MH-negative swine ($P < 0.05$; two-tailed *t* test) (fig. 2). However, there is considerable variability in this variable, with values occurring within 1 SEM of the mean for MH-negative swine in some MH-positive muscle and

values occurring within 1 SEM of the mean for MH-positive in some MH-negative muscle.

DISCUSSION

The present study extends to the Yorkshire/Duroc cross of swine the association of the C-to-T point mutation in nucleotide 1843 of the gene encoding the ryanodine receptor with MH susceptibility. In the Yorkshire/Duroc swine, inheritance of two mutated alleles appears to be necessary for MH susceptibility, in agreement with a recessive pattern of inheritance. However, even when both alleles are inherited, this mutation is not sufficient to elicit the MH syndrome at all times, despite potentially lethal anaesthesia. Instead, there appears to be an important modulating factor of the MH syndrome that must be present for full expression of the MH syndrome or, if it is absent, the progression of events resulting in the syndrome is interrupted and the syndrome is not observed, as was the case in three of the four non-rigid MH swine. These four swine were definitely MH susceptible, as they had previously exhibited rigidity in the barnyard challenge.

Unlike one other strain of swine [26] in which the heterozygotes exhibited an intermediate *in vitro* contracture response to halothane and another [27] in which the heterozygotes exhibited a positive contracture response by the European MH Group procedure [29], the Yorkshire/Duroc cross heterozygotes have about the same *in vitro* contracture response to halothane as homozygous normals. It should be noted that neither of the previous studies [26, 27] truly genotyped the swine, but relied instead on phenotypic information to establish a "genotype" indirectly. The mean of the resting CK concentrations was slightly greater in the Yorkshire/Duroc heterozygotes, but the phenotypes of the heterozygotes and the homozygous normals would be impossible to distinguish clearly using CK values, contracture tests, or both. Similarly, the mean of the values of the threshold of Ca^{2+} -induced Ca^{2+} release was normal for heterozygotes. A recent study has reported that heterozygotes form a metabolically distinct phenotype that is more closely related to controls than to homozygote MH susceptibles [30]. In agreement, the prolonged *in vivo* challenge with halothane and suxamethonium in the Yorkshire/Duroc heterozygotes did not result in any clinical variables differing statistically from controls. Two heterozygotes were identical to controls for all clinical variables examined, whereas values in two others were slightly outside the control range. The heterozygotes were not within the range of homozygous MH susceptibles exhibiting rigidity for any of the clinical variables examined. Therefore, although a much larger population might reveal slight but statistically significant differences between heterozygotes and controls, the heterozygotes in the present study could not be considered MH susceptibles.

Four swine homozygous for the MH mutation failed to exhibit rigidity, despite an extreme challenge with halothane and suxamethonium. While the base excess was negative for all four non-rigid MH

swine, three values were not remarkable relative to those in four of the five rigid MH swine. In addition, two of the non-rigid MH swine exhibited no signs of muscle breakdown (normal pH and lactate values), a third had values only slightly greater than those in controls, and none had accelerated heart rates. These non-rigid MH swine had previously demonstrated rigidity during a brief barnyard challenge with halothane alone and were, therefore, capable of being triggered at least some of the time. The findings in the four non-rigid MH swine would support a modifying factor that determines whether an MH episode can occur, and its severity. Although initial temperature has been suggested to be one potential modifying factor for the MH syndrome [25], the initial temperatures were the same for these four non-rigid MH-positive swine as for the swine exhibiting a full MH syndrome in the present study. Other potential modifying factors are free fatty acids [11], inositol 1,4,5-trisphosphate [31, 32] and an abnormality in the antioxidant defense system [33].

Studies of the age-dependence of the porcine stress syndrome have demonstrated that free fatty acid production parallels the acquisition of susceptibility to the syndrome [8]. A modulator gene does not have to be inherited as a defect. It may be a dormant normal gene product, such as a triglyceride lipase, that is turned on indirectly by the ryanodine receptor mutation (in swine). The porcine mutation results in substitution of Cys⁶¹⁵ for Arg⁶¹⁵ [15]; cysteine residues on proteins are the major sites for covalent attachment (acylation) of fatty acids [34]. Therefore, this could be a crucial site binding fatty acids which would then greatly enhance the response to halothane [11].

In contrast to the *in vivo* challenge, the *in vitro* contracture test conducted on tissue removed immediately before the *in vivo* challenge did detect the presence of the MH defect in all cases in the present study. Therefore, the contracture test is not modulated or environmentally responsive. However, based on a larger population (19 MH-negative and 27 MH-positive), we have estimated a sensitivity of 96 % and specificity of 95 % (one false positive and one false negative) for the North American MH Group procedure in the Yorkshire/Duroc swine. We caution that the same contracture test criteria may not be suitable for other strains of swine. The threshold of Ca^{2+} -induced Ca^{2+} release values for the MH-positive swine not exhibiting rigidity, as a group, were the same as those for the rigid MH-positive swine. While this finding would suggest that the threshold of Ca^{2+} -induced Ca^{2+} release was also independent of the MH syndrome modifying factor, the wide range of values in both groups and the wide range of modulation of this variable within individuals with other muscle disorders [35] make this an unreliable predictor of MH susceptibility.

Some strains of swine might express the factor modifying the response to anaesthetics more consistently than others and this may account for the strain-to-strain variation in the expression of the MH syndrome [5]. The Yorkshire/Duroc MH-susceptible swine are somewhat less responsive than some other strains. Our findings in the Yorkshire/

Duroc swine are the first to suggest that the expression of the MH syndrome in homozygous abnormal individuals in this strain can vary in the same manner as observed in humans with MH. One major difference between human MH and porcine MH is the pattern of inheritance. While both exhibit an autosomal inheritance, MH is inherited as a dominant disorder in humans and recessive disorder in swine. A second major difference is that all porcine MH has been suggested to result from a single genetic defect in the ryanodine receptor [15, 16] and this was verified for the strain of swine in the present study. In contrast, an analogous defect in humans is rarely inherited [36] and, if so, it is inherited as a single allele in MH humans. Also, genetic heterogeneity has been observed in humans, suggesting that genes other than the Ca^{2+} release channel can cause MH [37–41].

These findings in the Yorkshire/Duroc strain of swine suggest that inheritance of a single copy of the genetic mutation in the ryanodine receptor in the Yorkshire/Duroc swine does not significantly alter muscle function in the absence of anaesthetics, or the response to MH triggering agents. Inheritance of the porcine mutation as a single allele causative of MH in humans is inconsistent with the present and other studies in swine. Therefore, species-related differences in the Ca^{2+} release channel function or expression must be invoked to explain human MH. Alternatively, the possibility exists that the porcine mutation, when inherited as a single allele, in humans may not cause MH.

Inheritance of two abnormal alleles does result in abnormal muscle function in these swine in both the absence (increased CK values) and presence of anaesthetics. The absence of the MH syndrome in a subpopulation of swine homozygous for the MH defect, even with a prolonged halothane and suxamethonium challenge, suggests that swine may be used to study a modifying factor responsible for the variability of the syndrome in MH-susceptible humans. The outcome of the halothane and caffeine contracture tests appears to reflect the presence of the MH mutation independent of the modifying factor.

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PRESURGICAL ANALGESIA IN CHILDREN SUBJECTED TO HYPOSPADIAS REPAIR

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SUMMARY

We have examined the use of different pre-surgical analgesic techniques in 30 children aged 2–10 yr undergoing repair of hypospadias. They were allocated randomly, in a double-blind, placebo-controlled study, to receive one of three analgesic techniques ($n = 10$): lumbar extradural analgesia using 0.5% bupivacaine 1 mg kg^{-1} plus morphine $50 \text{ } \mu\text{g kg}^{-1}$; morphine $100 \text{ } \mu\text{g kg}^{-1}$ i.m.; or subpubic block using 0.5% bupivacaine 0.5 mg kg^{-1} for each side. Analgesics were given after induction of anaesthesia and before the start of surgery. Children given extradural analgesia had more stable haemodynamic variables and smaller catecholamine concentrations and needed less anaesthetic supplementation, with no analgesic requirement in the postoperative period. Nausea or vomiting were not reported in children given subpubic block. (Br. J. Anaesth. 1993; 71: 418–421)

KEY WORDS

Anaesthetic technique: extradural, regional. Analgesia: pre-emptive, postoperative.

Hypospadias repair produces severe postoperative pain which may be treated at various levels in the nervous pathways involved. Local anaesthetics are the most potent inhibitors of pain, acting at either the axonal target of the peripheral nerves [1] or the level of the dorsal horn of the spinal cord [2].

Noxious stimuli generate prolonged post-stimulus sensory disturbances in the form of pain, hyperalgesia and allodynia [3]. Pre-injury block has been shown to reduce this hypersensitivity [4]. The concept of pre-emptive treatment of postoperative pain has been used at the axonal level of the peripheral nerves [1] and the spinal cord level [2, 5]. Pre-emptive treatment of acute pain at brain level is still unproven.

The purpose of this study was to test the effect of different pre-surgical analgesic strategies in children subjected to hypospadias repair.

PATIENTS AND METHODS

We studied children aged 2–10 yr with penile hypospadias undergoing repair operations. Apart from this minor congenital deformity, all children were healthy. Exclusion criteria included a history of allergy to local anaesthetics, mental retardation and anomaly or disease in the lumbar vertebrae or pelvic

bones. The randomized, double-blind, placebo-controlled study was approved by the local responsible authorities and explained to the parents or guardians before written consent was obtained. The anaesthetist, the surgeon and recovery room nurse were blinded to the type of medication given.

Children aged 2–5 yr were given diazepam 1 mg orally the night before surgery and 2 h before anaesthesia; those older than 5 yr received 2 mg . They were admitted to the operation room early in the morning and a heparinized venous blood sample was obtained for measurement of plasma catecholamine concentrations. Anaesthesia was induced with thiopentone 5 mg kg^{-1} and atropine 0.02 mg kg^{-1} and tracheal intubation was facilitated by i.v. administration of suxamethonium 1 mg kg^{-1} . Anaesthesia was maintained with 66% nitrous oxide in oxygen supplemented with halothane. Heart rate and arterial pressure were maintained in a quasi-steady state by adjusting the halothane concentration. Neuromuscular block was produced with vecuronium (initial dose 0.1 mg kg^{-1}). Central venous cannulation was performed via the right internal jugular or subclavian veins, for blood sampling and fluid administration.

After induction of anaesthesia and before the start of surgery, analgesia was given by one of three techniques: lumbar extradural, i.m. or subpubic. The solutions were prepared outside the operating room so that they could not be recognized by the anaesthetist. All injections were performed by the same person, and all the children received injections in all three sites (lumbar extradural, i.m. and subpubic space). Active drug was given in only one site, saline being given at the remaining two sites. Surgery started after 25 min.

Thirty children were allocated randomly to three groups. In group I, lumbar extradural analgesia was produced in 10 children by 0.5% bupivacaine 1 mg kg^{-1} mixed with morphine $50 \text{ } \mu\text{g kg}^{-1}$ given via the L2–3 space. In group II, morphine $100 \text{ } \mu\text{g kg}^{-1}$ was given i.m. in the gluteal region in 10 children. In group III (10 children), bilateral subpubic block was performed [1] using 0.5% bupivacaine 0.5 mg kg^{-1} for each side.

Monitoring comprised heart rate, continuous

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TABLE I. Clinical data and anaesthetic time in children subjected to hypospadias repair (mean (range))

Group	Age (yr)	Body weight (kg)	Height (cm)	Duration of anaesthesia (min)
I: Lumbar extradural analgesia	5.6 (3–7)	19.0 (14–22)	106.7 (93–120)	111 (80–140)
II: I.m. morphine	6.5 (3–9)	21.2 (14–28)	115.6 (90–134)	109 (60–140)
III: Subpubic block	5.1 (2–10)	17.8 (10–30)	102.1 (75–138)	120 (65–165)

three-lead ECG, non-invasive arterial pressure and pulse oximetry. Central venous blood samples were obtained for measurement of plasma concentrations of adrenaline, noradrenaline and morphine. Samples for measurement of catecholamines were obtained 30 min after the start of surgery, at the end of surgery and 60 min after anaesthesia. Samples for morphine were obtained 2, 5, 10, 20, 30, 45, 60, 75, 90, 120 and 240 min after the injection of analgesic.

The senior recovery room nurse assessed all children according to an observational scoring system [6] using crying, movement, agitation, posture and localization of pain as scoring criteria—each scored 0–4, to give a range of 0–20 for a summed total score. Vomiting and nasal itching were recorded. Midazolam 0.1 mg kg⁻¹, alone or with fentanyl 1.0 µg kg⁻¹ or pethidine 1.0 mg kg⁻¹, was given to children with high scores. The children were observed in the ward for pain, vomiting and nasal itching.

Plasma concentrations of catecholamines and morphine were measured by HPLC using electrochemical detection at 750 mV and 700 mV, respectively. The extraction and assay of catecholamines have been described previously [7]. Sodium metabisulphite was used as antioxidant and the mobile phase consisted of acetonitrile/monosodium acid phosphate 0.1 mol litre⁻¹ (10:90), 1-octane sulphonic acid 4 mmol litre⁻¹, EDTA 0.1 mmol litre⁻¹, sodium chloride 0.01 mol litre⁻¹ and pH adjusted to 3. Calibration curves were constructed daily for each catecholamine using dihydroxybenzylamine as internal standard. The lower limits for detection were 0.06 and 0.07 ng ml⁻¹ for noradrenaline and adrenaline, respectively. The technique of morphine extraction and assay has been described elsewhere [8]; we used toluene and not benzene in the extraction process. A calibration curve was constructed daily using nalorphine as internal standard. The lower limit for detection of morphine was 2.5 ng ml⁻¹.

Data were analysed using an SPSS statistical program. Intergroup comparison was performed using the Mann–Whitney *U* test. Non-parametric variables were analysed by Friedman test for intergroup difference and by Wilcoxon rank-sum test for two-group difference. *P* < 0.05 was taken as significant. The statistical software Pharm/SCS was used for analysis of pharmacokinetics. Plasma concentration–time data were described best by a two-compartment model with first order absorption. The total area under the curve (AUC) was derived using the trapezoid rule.

TABLE II. Mean perioperative heart rate and arterial pressure (AP) in children subjected to hypospadias repair. Significant differences (*P* < 0.05): * compared with group I; † compared with group II

	Before induction	During anaesthesia		Recovery	
		30 min	End	30 min	60 min
Heart rate (beat min ⁻¹)					
Group I	119	133	101	89	98
Group II	125	122	103	96	102
Group III	112†	125	117*†	101	102
Systolic AP (mm Hg)					
Group I	111	104	106	102	99
Group II	113	106	120*	111*	108*
Group III	111	113*	115	112	112*
Diastolic AP (mm Hg)					
Group I	60	53	60	53	51
Group II	59	53	57	58	54
Group III	60	62*	60	65*	60

TABLE III. Number of children who required an analgesic, sedative, or both, suffered from vomiting, or developed nasal itching during their stay in the recovery room or in the ward after hypospadias repair, in groups I (lumbar extradural block), II (morphine i.m.) and III (subpubic block). NR = Not reported

	Recovery room			Ward		
	I	II	III	I	II	III
Analgesic–sedative						
Midazolam 0.1 mg kg ⁻¹	1	3	1	0	0	0
Midazolam 0.1 mg kg ⁻¹ + pethidine 1 mg kg	0	1	4	0	0	0
Midazolam 0.1 mg kg ⁻¹ + fentanyl 1 µg kg ⁻¹	0	2	1	0	0	0
Vomiting	3	4	NR	2	1	NR
Nasal itching	4	NR	NR	3	4	NR

RESULTS

Clinical data and duration of anaesthesia are shown in table I. Heart rate increased significantly by the end of anaesthesia in children in group III (subpubic block) (table II). However, during recovery there was no significant difference between the three groups. Arterial pressure was least during anaesthesia and recovery in the lumbar extradural group (group I) (table II). Perioperative plasma concentrations of adrenaline did not differ between groups, while plasma concentration of noradrenaline was greater in the morphine group (group II) than in the two other groups before anaesthesia and during surgery. During recovery there was no significant difference in plasma noradrenaline concentration between groups.

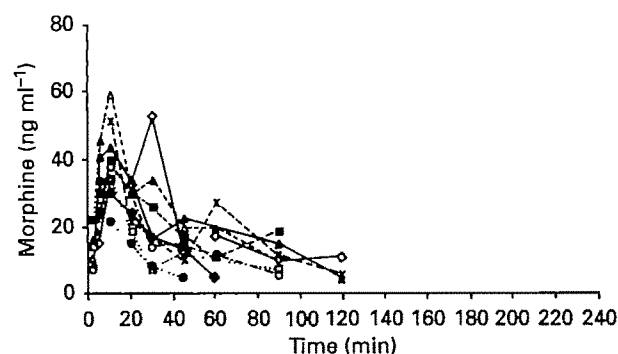


FIG. 1. Individual plasma morphine concentrations after lumbar extradural administration of morphine 0.05 mg kg^{-1} in nine children undergoing hypospadias repair.

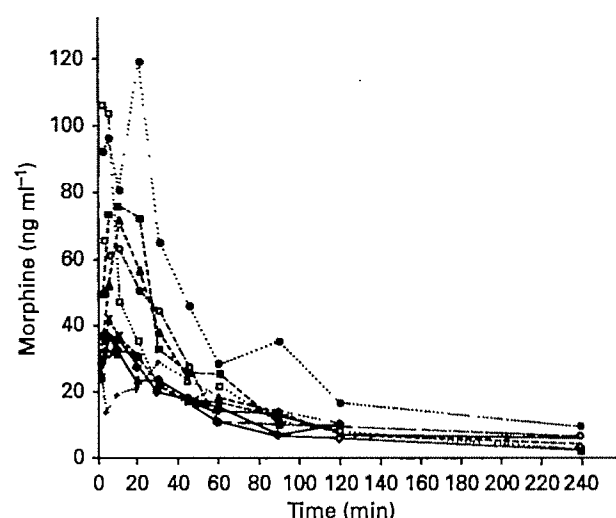


FIG. 2. Individual plasma morphine concentrations after i.m. administration of morphine 0.1 mg kg^{-1} in 10 children undergoing hypospadias repair.

TABLE IV. Pharmacokinetic variables (mean (SD)) of morphine given extradurally (0.05 mg kg^{-1}) or i.m. (0.1 mg kg^{-1}) to children undergoing hypospadias repair. K_a = absorption constant; C_{max} = maximum plasma concentration; t_{Cmax} = the time at which C_{max} was reached; AUC = the area under the plasma concentration-time curve. * Significant difference between groups ($P < 0.05$)

	Extradural morphine ($n = 9$)		I.m. morphine ($n = 10$)
K_a	0.51 (0.38)	*	0.78 (0.26)
C_{max} (ng ml^{-1})	42.7 (10.2)		61.6 (31.9)
t_{Cmax} (min)	11.7 (7.1)	*	6.6 (3.3)
AUC ($\text{ng ml}^{-1} \text{ min}^{-1}$)	2716 (1115)	*	3385 (424)

Two children in the lumbar extradural group needed supplementation with 0.5% and 1% halothane for 40 and 5 min, respectively; four children in the morphine group required 0.5–1% halothane for a mean duration of 50 min. Most children ($n = 9$) in the subpubic block group needed supplementation with halothane in greater concentrations (0.5–2%) for a mean duration of 60 min.

During recovery, the mean observational scores in groups I, II and III were 0.8, 2.8 and 4.6, respectively (significant differences between groups),

but there was no significant difference in SpO_2 values between groups. Only one child in the lumbar extradural group (I) needed sedation, while more than 50% of the other children required sedation and analgesia (table III). Vomiting was reported in groups I (lumbar extradural) and II (morphine), but nasal itching occurred only in group I (table III).

In the ward, vomiting was reported in two children who received lumbar extradural analgesia and in one child in the morphine group. Nasal itching was observed in both these groups (table III).

Plasma concentrations of morphine are displayed in figures 1 and 2 for children in groups I (lumbar extradural) and II (morphine), respectively. The pharmacokinetic variables are calculated in table IV. There was no significant difference in peak plasma concentrations (C_{max}) between the two groups. The time at which C_{max} was reached (t_{Cmax}) was significantly delayed in group I children. However, the area under the plasma concentration-time curve (AUC) was less in that group compared with children given morphine.

DISCUSSION

We have shown that pre-surgical administration of lumbar extradural block was associated with a more stable intraoperative course than was achieved with pre-surgical administration of subpubic block or i.m. morphine. Heart rate and arterial pressure were relatively more stable, while fewer children required halothane supplementation and for a shorter duration. These findings were not unexpected, as the combination of local anaesthesia and a strong opioid such as morphine at the neuroaxial level protects against noxious stimuli.

The outcome measures in the postoperative period involved observational scoring and the total sedative and analgesic requirements. Pre-verbal children were excluded and we relied on behavioural observations as being more objective and independent of verbal skills. We encouraged the nurse to communicate with the children to accommodate changes in pain behaviour which may probably occur in older children. The observational score was smallest in children given lumbar extradural analgesia, and only one child needed sedation in the recovery room. Nausea and vomiting were not reported in children after subpubic block. This is an advantage of this simple technique.

The degree of postoperative analgesia was not a function of the plasma concentration of morphine. Two hours after extradural morphine administration, it was possible to detect appreciable concentrations of morphine in only three children, while after 4 h no morphine could be detected in any child in this group. I.m. administration of morphine produced greater plasma concentrations and for a longer duration than after extradural administration, yet there was no significant difference in postoperative outcome measures. This may be attributable to the concomitant administration of bupivacaine and deposition of morphine near opioid receptors in the neuraxis in the extradural group.

The purpose of pre-emptive analgesia is to prevent

encoding of the painful stimulus in the nervous system. This encoding has been delayed in our children given lumbar extradural analgesia, by administration of local anaesthetic and a strong opioid, resulting in satisfactory outcome measures during surgery and in the postoperative period. This cannot be claimed in children given subpubic block because it is not certain if all nerves can be blocked with subpubic injection.

The limitation of this study is that we have not administered analgesia randomly at the start of surgery or after the completion of the repair and thus this is not a definitive study of pre-emptive analgesia. However, pre-surgical analgesia using lumbar extradural block with a local anaesthetic and opioid produced satisfactory surgical and postoperative outcome measures in our children. Subpubic block and parenteral morphine produced moderate outcome measures.

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REVIEW ARTICLES

HYALURONIDASE

D. WATSON

In 1929, Duran-Reynals investigated a spreading factor found in certain strains of streptococcal bacteria [4]. This factor was discovered to be an enzyme acting predominantly on hyaluronic acid and it was subsequently named hyaluronidase. The enzyme has also been found in the ox spleen, iris and cornea and snake and honey bee venoms [5, 23]. However, there are differences between these enzymes, as they possess different pH optima. Hyaluronidase is used to improve the spread of local anaesthetic solutions through tissue planes. The predominant substrates for hyaluronidase are hyaluronic acid and, to a lesser extent, chondroitin sulphates.

Composition of connective tissue

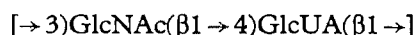
The major component of connective tissue is collagen. Depending on the site of the structure and its function, there are variable arrangements of the collagen bundles. The dura mater, for instance, consists of both collagen and elastin fibres; on both inner and outer surfaces are layers of flattened fibroblasts [39].

Biochemistry

The “ground substance” which surrounds cells comprises the proteoglycans. These consist of proteins bound covalently to the glycosaminoglycans hyaluronic acid (1% protein binding), chondroitin-4-sulphate and chondroitin-6-sulphate. The relative molecular mass of such a proteoglycan molecule is 2×10^6 Da (1 Da = one-twelfth the mass of a carbon-12 atom).

Approximately 20–250 of these proteoglycan molecules form an aggregate with hyaluronic acid. Hyaluronic acid is synthesized by fibroblasts and their derivative cells and, to a degree, chondrocytes. It is a linear polymer of alternate glycosidic-linked monosaccharides, D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose (fig. 1) [3, 11].

The repeating subunit of hyaluronic acid (disaccharide component) may be shown by the notation [11]:



where GlcNAc = 2-acetamido-2-deoxy-D-glucose, GlcUA = D-glucuronic acid and the arrows and

numbers, respectively, refer to the glycosidic-linkage regions between monomer moieties forming the unbranched, heteropolysaccharide chain.

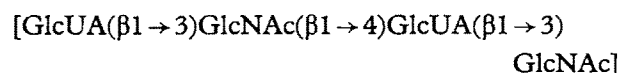
Physical properties of ground substance

Hyaluronic acid is found within connective tissues such as Wharton's jelly in the umbilical cord, skin, aorta, vitreous humour of the eye, joint cartilage and the nucleus pulposus, in addition to the fluid of synovial joints. A few of these are examples of protein glycosaminoglycan gels. Hyaluronic acid, being a polyanion, has its carboxyl groups completely ionized at pH 7.0 and is therefore highly soluble in water [19].

The viscosity of synovial fluid is directly attributable to the hyaluronic acid content and its inherent hygroscopic properties. Chondroitin sulphates are present in more dense structures such as ligaments and tendons and are particularly important in the structure of articular cartilage.

Action of hyaluronidase

Mammalian hyaluronidase (trade name Hyalase; CP Pharmaceuticals Ltd, Wrexham—a subsidiary of Fisons plc, Loughborough) is more correctly termed hyaluronoglucosaminidase (an endoglycosidase). This enzyme liquefies the interstitial barrier, present between cells, by cleaving the β -1,4 glycosidic bond between 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid. After prolonged action of the enzyme, the tetrasaccharide is formed:



This is a reversible and short-lived depolymerizing action which is pH- and temperature-dependent. However, breakdown products are excreted in the urine [11].

Therefore, under the action of hyaluronidase the normal, high viscosity and lubricating ability of hyaluronic acid is lessened and this improves the diffusion of injected substances within tissue planes. However, the spread of substances across collagenous barriers is not facilitated.

Commercial availability

Commercially available hyaluronidase is of ovine origin and is supplied for clinical use as a white,

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KEY WORDS

Anaesthetics, local. Enzymes: hyaluronidase.

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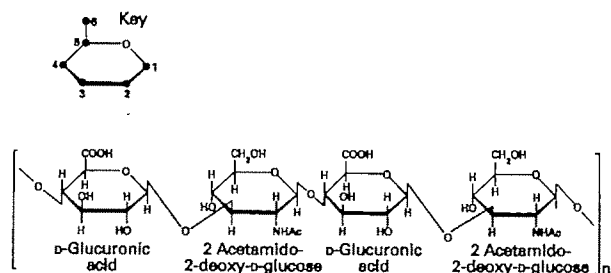


FIG. 1. Hyaluronic acid: tetrasaccharide segment of the carbohydrate chain, showing basic structural elements of glycosaminoglycans.

freeze-dried, water soluble powder containing 1500 i.u. (BP) per ampoule. It also contains a trace of tyrosine (less than 0.15 mg/unit of hyaluronidase).

There are cases of allergy recorded in the literature, and hyaluronidase has an apparent role in the massive increase in capillary permeability seen in anaphylactic reactions [34]. Therefore, caution is advised in atopic individuals [37].

The United Kingdom supplier to the health profession is CP Pharmaceuticals Ltd, but until 1985 makers included Evans Medical Ltd and, previously, Bengel Ltd. The sales figures for this period give an idea of the importance of hyaluronidase in medical practice. In 1985 there were 82000 ampoules sold and in 1990, 110000 ampoules (Marketing Department figures). There is also a stable injectable solution, Wydase (R) (pH 6.4–7.4), which must be stored at 2–8 °C. The recommended dose is 1500 i.u., regardless of volume injected.

The product licence for hyaluronidase has been extended recently for use with s.c. and i.m. injections. In patients in whom there has been extravasation of cytotoxic substances, hyaluronidase has a place in limiting destruction of subcutaneous tissues [9].

There is also a topical preparation of hyaluronidase with heparin, for use on bruised areas of skin to reduce the inflammation and thrombophlebitis associated with needle puncture sites and the oedema associated with haemorrhoids. This mixture is in an ointment form under the trade name Lasonil (Bayer U.K. Ltd). This product contains 150 i.u. of hyaluronidase and heparinoid 50 μg^{-1} (a semi-synthetic, sulphated derivative of heparin).

Past uses

The major problem with previous work has been the lack of project design and statistical analysis [12, 14, 17, 26, 31]. Recently, hyaluronidase was used, with little obvious success, in patients suffering from early myocardial infarction [32]; thrombolytic agents such as streptokinase have superseded hyaluronidase in this condition.

Midazolam has been given i.m. for premedication, with hyaluronidase [13]. Unfortunately, the midazolam was extremely irritant and painful.

Although hyaluronidase improves the absorption of fluids administered s.c., the advance of safe, reliable venous access has removed the need for hypodermoclysis. However, it is historically interesting to note that 1000 ml of fluid may be given over

about 1.5 h by this route when hyaluronidase 1500 i.u. is added to the solution; the hydrostatic pressure required is about 100 cm H_2O [10].

Hyaluronidase has been used to reduce synovial fluid viscosity in diseased joints and topically in combination with local anaesthetics for tracheal intubation [14, 31]. Kurzrok, Leonard and Conrad [17] investigated the association of hyaluronidase production, by malignant cells, with the spread of mesothelioma of the pleura and peritoneum.

Local anaesthetic uses

Duran-Reynals was the first to investigate the "spreading ability" of hyaluronidase combined with plain local anaesthetics [6]. In order to observe the effect better, he used India Ink as a marker. When the tissues were "massaged" there was a greatly increased spread of the dye, in keeping with its action in reducing the integrity of ground substance. Further studies by Forbes and colleagues [10] and DeFelice and colleagues [2] showed conflicting results. In the latter case, the use of the marker Phenol Red in ethanol may have altered the activity of the enzyme and thus limited the spread of the marker.

Kirby, Eckenhoff and Looby studied the combination of adrenaline, hyaluronidase and local anaesthetics and suggested that adrenaline reduced the uptake, but not the spread, of local anaesthetics administered s.c. [16].

Many combinations of local anaesthetics and hyaluronidase have been used in almost every possible situation. These have included dental procedures, fractures and regional blocks [38]. However, there are no satisfactory data for statistical interpretation in most of the earlier studies [8, 25, 29, 36].

The use of hyaluronidase to promote regression of oedema in acute thrombosed haemorrhoids improves operating conditions and enhances spread of the injected local anaesthetic [35]. The enzyme allows smaller volumes of local anaesthetics to be used and, when combined with adrenaline, gives prolonged postoperative analgesia. This is not a surprising finding, in view of the evidence by previous investigators [2, 8]. The lessened tumescence of the soft tissues during digital nerve blocks, using plain solutions, was used to advantage by Phalen [30].

Present uses

Plastic surgeons still use hyaluronidase, but its importance may be declining [22]. The advantage of the combination of hyaluronidase and plain local anaesthetics was to provide larger areas of donor skin grafts without obscuring tissue landmarks for the same volume of local anaesthetic administered s.c. Lewis-Smith demonstrated this application and used fluorescein to monitor the spread of lignocaine injected s.c. in forearm grafts [21]. This repeated previous work by Duran-Reynals in 1929 [4], but used more modern methods.

The major present day use of hyaluronidase is in the specialty of ophthalmology [24]. The early work of Atkinson and Lebensohn still influences the use of hyaluronidase in eye surgery [1, 18]. Nicoll and

colleagues gave a good account of the continuing place of hyaluronidase in retrobulbar blocks for cataract surgery [28]. The apparent lack of toxic reactions after retrobulbar block was shown in an audit of 6000 blocks [27]. This safety was confirmed by Rosenblatt, May and Barsoumian [33]. However, they found that severe reactions, including cardiac arrest, still occurred when hyaluronidase was not used, and therefore improving the spread of the local anaesthetic did not influence subsequent reactions.

More recent work from North America has demonstrated the advantage of buffering the acidic injectate of lignocaine-adrenaline-hyaluronidase with sodium bicarbonate [7]. This improved the comfort of the local anaesthetic infiltration and made day-case ophthalmic procedures more acceptable. However, buffering of plain bupivacaine solutions, containing hyaluronidase, with sodium bicarbonate to give a stable pH of 6.8 resulted in a greater requirement for supplementary peribulbar block [20].

Keeler and colleagues studied the place of hyaluronidase in axillary brachial plexus blocks and showed that the addition of this agent, together with adrenaline, decreased the duration of anaesthesia [15]. Whether hyaluronic acid is actually present within the axillary sheath was not discussed. The number of patients in both groups studied was small and there were a large number of failures. The only apparent difference between the study groups served merely to confirm that there was more rapid regression of anaesthesia when hyaluronidase was used. This is certainly a disadvantage for the management of postoperative pain.

The future

The greatest use of hyaluronidase will continue to be in the specialty of ophthalmology. Improving and encouraging patient acceptance of local anaesthesia may be increased if the pain of injection is lessened [7]. This may obviate general anaesthesia and, in the long term, have cost benefits.

Other areas in which hyaluronidase may have applications could include local anaesthetic nerve blocks for postoperative pain relief. The use of local anaesthetic-hyaluronidase combinations in pain relief for high ligation in varicose vein surgery or hernia surgery may be worthy of further study.

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INTERPLEURAL ANALGESIA

D. F. MURPHY

The technique of interpleural analgesia for patients with acute or chronic pain has not yet been the subject of critical review. As there is now an extensive list of published studies of the use of this technique, it seems timely to attempt to define the place of interpleural analgesia and anaesthesia in clinical practice. This article attempts to review anatomical and pharmacological considerations, in addition to evaluating the efficacy of interpleural analgesia after different types of surgery and in chronic pain management.

BACKGROUND

Although block of the intercostal nerves is not new, interest in this technique was revived when Nunn and Slavin re-examined the anatomy of the intercostal space in cadavers [48]. These authors deduced that an injection deep to the posterior intercostal membrane would allow the injected solution to spread from the site of injection to adjacent intercostal spaces. This idea led O'Kelly and Garry to describe a technique of multiple intercostal nerve blocks using a single intercostal injection in a patient with multiple unilateral fractured ribs [49]. The first series of patients in whom this technique was used was described by Murphy in 1983 [44, 45], who used it in patients with multiple fractured ribs and in postoperative patients after gallbladder and kidney surgery. One year later, Reiestad and Kvalheim [54] published their results of continuous intercostal nerve block for postoperative pain relief and presented their modification of the technique, which is now termed "interpleural analgesia" [32].

ANATOMICAL CONSIDERATIONS

Recent studies have shown that there is considerably more variability in the anatomy of the intercostal space than is suggested in the classic textbooks of anatomy [3] and neural block [84]. Classically, the intercostal muscles are described in three layers (figs 1, 2). The external intercostal muscle originates from the tubercles and the lower borders of each rib and is directed obliquely downwards and forwards to be inserted into the upper borders of the adjacent ribs

below. The internal intercostal muscle originates from the lower border of each rib and costal cartilage and is directed obliquely backwards to be inserted into the upper borders of the adjacent ribs below. The innermost layer of muscles, which corresponds to the transversus abdominis muscle in the abdomen, is composed of a group of muscles, the transversus thoracis group. The most important of these, the intercostalis intimus, runs obliquely downwards and backwards from the inner edge of the ribs to the upper borders of the adjacent ribs. Some fibres may bridge more than one intercostal space to be inserted into the 2nd or 3rd adjacent rib. The intercostal nerve is classically described as running under the shelter of the intercostal groove, and is situated below the intercostal vein and artery.

Nunn and Slavin [48] performed detailed studies in six cadavers subjected to postmortem. At the 6th intercostal space, 7 cm from the posterior midline, the ribs were found to have an average thickness of approximately 9 mm. At this point the external intercostal muscle was of variable thickness, but was well developed and bound internally by the posterior intercostal membrane—the aponeurotic extension of the internal intercostal muscle. In contrast to this sturdy membrane, the innermost intercostal muscle, the intercostalis intimus muscle was "a flimsy structure composed of several fascicles through which injected India ink passes freely to reach the subpleural space". The nerves, arteries and veins were consistently found in the tissue plane deep to the posterior intercostal membrane and superficial to the intercostalis intimus muscle, with no fixed relationships to the ribs above or below. The intercostal nerves were found to run "as three or four separate bundles with no single neural sheath" and with considerable variation in relationship to and size compared with associated intercostal arteries and veins. Studies by Hardy [26] on the anatomical variation in position of the nerves to the ribs concur with the findings of Nunn and Slavin. In 30 cadavers, the 2nd to 11th intercostal nerves were dissected and were found to occupy the classically described subcostal position in only 17% of cases.

As the intercostal nerves were found consistently to occupy the tissue plane deep to the posterior intercostal membrane and superficial to the intercostalis intimus muscle, initial attempts at con-

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KEY WORDS

Anaesthetic techniques, regional: interpleural analgesia.

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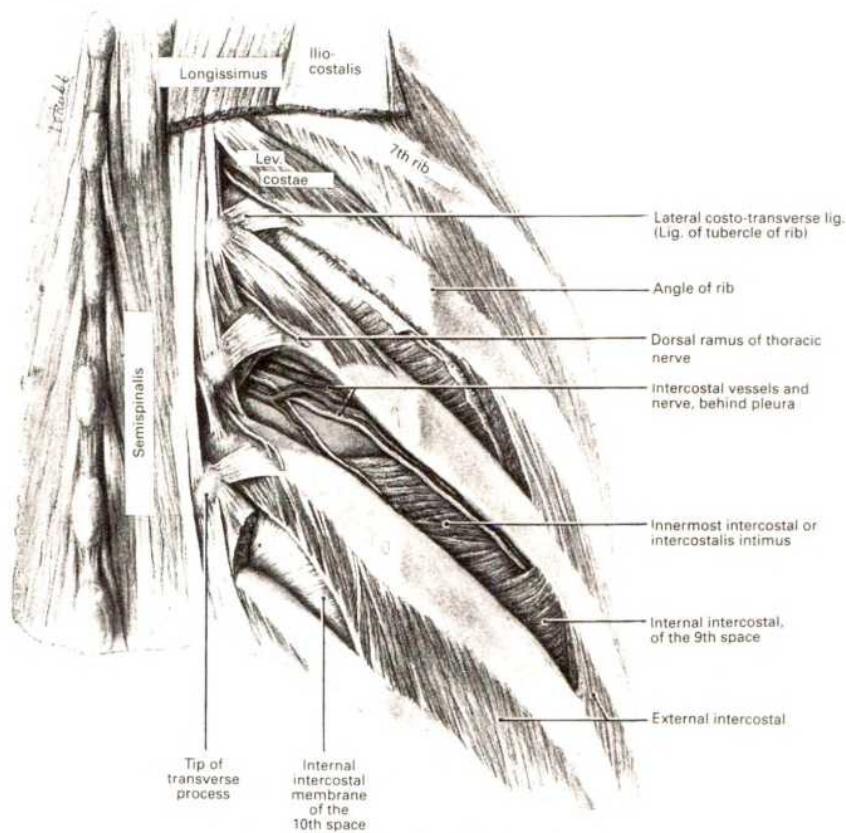


FIG. 1. Posterior end of intercostal space.

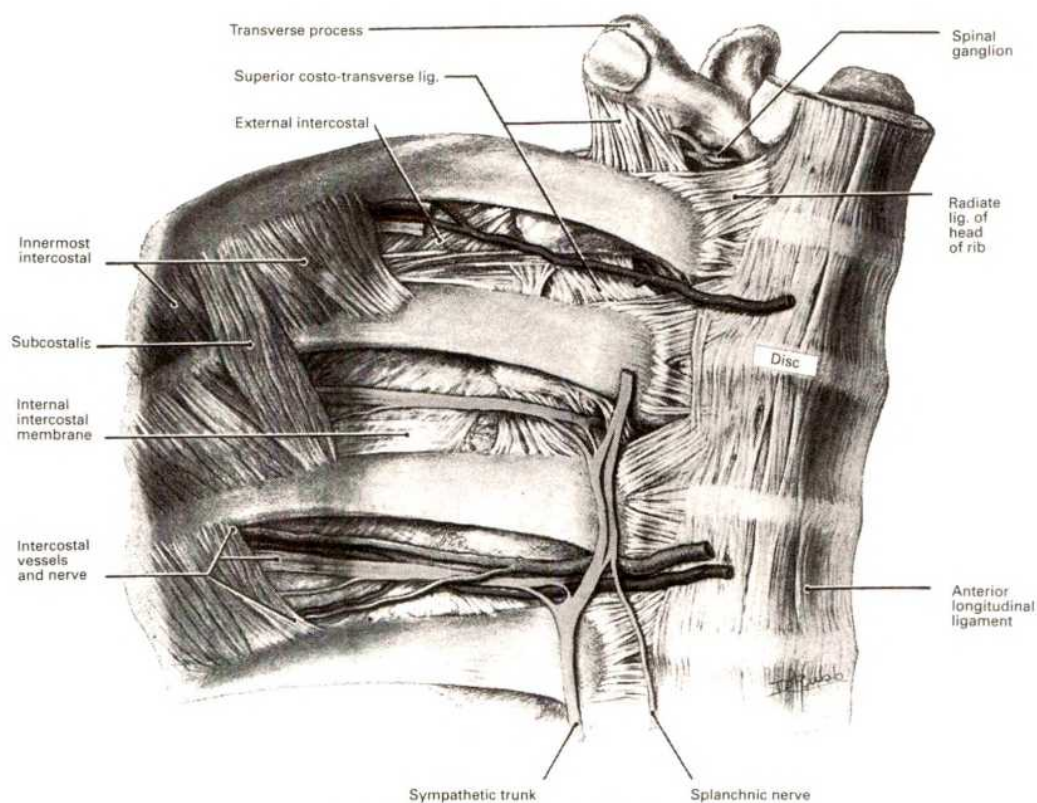


FIG. 2. Vertebral end of intercostal space.

tinuous intercostal nerve block were directed at location of this tissue plane and assessment of the degree of spread of solutions injected into this space. In a study in cadavers, Murphy [46] infused India

ink through an extradural catheter inserted into this tissue plane and examined the spread of the ink within the chest cavity after removal of the thoracic viscera. It was found that “by pushing the parietal

pleura forward, the dye spread easily, sub-pleurally, to reach intercostal spaces above and below the one cannulated". In only 50 % of the examinations did the ink spread medially to reach the paravertebral space. Similar results were found by Crossley and Hosie [14], who radiographically mapped the spread of iopamidol with lignocaine in 10 healthy volunteers. In contrast, Mowbray, Wong and Murray [42] found that spread of methylene blue into the paravertebral space occurred almost invariably and they believe that it is by this means that analgesia can extend over several dermatomes.

Few investigators have attempted to elucidate the mechanism of action of local anaesthetic agents injected between the parietal and visceral pleura. Studies in dogs [60] using somatosensory evoked potentials indicated that interpleural bupivacaine produces gravity-dependent intercostal nerve block, with no evidence of spinal or extradural block. Studies by Stromskag, Hauge and Steen [78], using computed tomography in patients receiving interpleural analgesia after different types of surgery, are in agreement with these findings. They suggested that interpleural local anaesthetics act by retrograde diffusion to reach the intercostal nerves.

TECHNIQUE OF CONTINUOUS INTERPLEURAL ANALGESIA

The principle of the technique is to deposit the local anaesthetic drug in a tissue plane which allows it to reach several intercostal nerves after a single injection. Two such tissue planes are available for this purpose: the tissue plane deep to the internal intercostal muscle but superficial to the parietal pleura, and that between the parietal and visceral layers of pleura.

Cannulation of the tissue plane deep to the internal intercostal muscle was described first, but this plane is more difficult to locate because of a lack of precise end-point for its determination. In this technique, a Tuohy needle is inserted with the bevel pointed medially within 7–8 cm of the spinous process. Location of the plane relies on insertion of the needle 5–8 mm deep to the rib. A characteristic "pop" may be felt as the aponeurosis is pierced. Passage of an extradural catheter into this space may be facilitated by directing the needle at a slight angle toward the midline. The catheter may be inserted 3–5 cm within this tissue plane with ease.

Cannulation of the tissue plane between the parietal and visceral pleura is accomplished in a similar manner and in a similar location, although the mid-axillary line also has been used [6]. When the posterior intercostal membrane is pierced, the trocar of the Tuohy needle is removed and a freely moving air-filled glass syringe is attached to the hub of the needle. The needle is then advanced using a "loss of resistance to air" technique until the pleural cavity is located. The extradural catheter is then inserted 5–6 cm [59]. Modifications to this technique have been described which aim to reduce the potential for air entrainment into the pleural cavity. These include use of a "hanging drop technique" [76] and use of a fluid-filled syringe

to detect negative pleural pressure [71, 89]. The catheter may also be inserted by the surgeon during operation when appropriate [38, 64, 65].

TAXONOMY

Because the techniques of intercostal and interpleural analgesia, as described above, do not differ in their proposed mode of action, the term "interpleural analgesia" is used henceforth to describe both approaches.

The terms "interpleural" and "intrapleural" have been used interchangeably to describe the space between the parietal and visceral pleura into which local anaesthetic is deposited. Covino [13] suggested that the term "interpleural" be used because "the catheter is located and the local anaesthetic solution deposited between the two layers of pleura rather than within the pleura". As pointed out by other authors, however, "parietal and visceral pleura are continuous around the hilar structures and the right and left pleural sacs are distinct. Therefore, an injection of local anaesthetic through a needle that has penetrated the parietal membrane, but not the visceral membrane, is intrapleural" [39]. It has been suggested that both prefixes be eliminated in the interest of simplicity and that the technique be described as "pleural block" [4]. In the absence of a consensus, the more common term of "interpleural analgesia" is used here.

CLINICAL STUDIES

The indications for which interpleural analgesia has been used are listed on table I. Most clinical studies have been performed in patients recovering from gallbladder or thoracic surgery. Less frequently, this technique has been used for pain relief in patients with multiple fractured ribs. Other indications are uncommon.

Pain relief after cholecystectomy

Studies in patients after cholecystectomy have consistently shown interpleural analgesia to be effective [7, 18, 21, 22, 30, 33, 34, 44, 45, 50, 59, 70, 80, 81, 87]. In a study by Murphy [44] of patients receiving 0.5 % bupivacaine 20 ml as required after cholecystectomy, 92 % and 76 % required no other supplementary analgesia in the first 24 and 48 h after surgery, respectively. Development of tachyphylaxis was the indication for opioid supplementation in

TABLE I. *Indications for interpleural analgesia*

Postoperative analgesia
Cholecystectomy [7, 18, 21, 33, 34, 44, 48, 59, 81, 87]
Thoracic surgery [28, 36, 38, 42, 43, 62, 63, 65, 67]
Renal surgery [32, 45, 55, 59, 82, 85]
Breast surgery [55, 59, 69]
Cardiac surgery [5, 6]
Abdominal surgery [17, 35]
Multiple fractured ribs [25, 45, 49, 61]
Cancer pain management [15, 16, 20, 58]
Acute herpes zoster [27]
Post-herpetic neuralgia [56, 58, 74, 75]
Pain in chronic pancreatitis [1, 58, 75]
Reflex sympathetic dystrophy [57]
Upper limb ischaemia [53]

most subjects. The mean duration of action of bupivacaine in this study was approximately 7 h.

VadeBoncouer and colleagues [87] compared interpleural bupivacaine with interpleural saline in a study of patients also receiving morphine via a patient controlled analgesia (PCA) device. On the first day after operation these patients received a single injection of 0.5 % bupivacaine 20 ml with adrenaline or 0.9 % saline 20 ml by random allocation. Their PCA morphine requirements were then evaluated over the succeeding 6 h. Patients receiving interpleural bupivacaine required significantly less morphine during this period compared with patients receiving saline. A similar reduction in PCA morphine requirement was found by Lee and colleagues [34], who administered interpleural bupivacaine or saline 4-hourly for 24 h. The mean morphine requirements over this period were 21.6 mg and 72.4 mg, respectively.

In a comparison of 0.25 %, 0.375 % and 0.5 % bupivacaine with adrenaline in patients after cholecystectomy, Stromskag and colleagues [81] found the quality of analgesia to be consistently good in all groups. The mean visual analogue pain score did not exceed 20 mm (0–100 mm scale) for all groups up to 4 h after injection. The duration of action was very variable, however (range 2–18 h). In comparing different volumes of interpleural bupivacaine, Stromskag, Minor and Lindeberg [80] found that 0.5 % bupivacaine 20 ml was similar in effect to 0.25 % bupivacaine 40 ml.

Laurito and colleagues [33] compared a continuous infusion of interpleural 0.25 % bupivacaine $0.125 \text{ ml kg}^{-1} \text{ h}^{-1}$ with intermittent bolus doses of 0.5 % bupivacaine 0.4 ml kg^{-1} and found the infusion to provide better pain relief.

Pain relief after thoracotomy

Although the benefits of interpleural analgesia after cholecystectomy have been demonstrated consistently, studies on its use after thoracotomy have been inconclusive. McIlvaine and colleagues [37, 38] reported the use of this technique in 14 children in whom interpleural catheters were inserted before chest closure and a continuous interpleural infusion of 0.25 % bupivacaine with adrenaline $0.5\text{--}1 \text{ ml kg}^{-1} \text{ h}^{-1}$ was used; opioid analgesia was available as required. These authors found that interpleural analgesia was effective and demonstrated

infusion of 0.25 % bupivacaine to 14 patients after thoracotomy and prescribed i.m. oxycodone as required. They found that all patients required oxycodone in doses which did not differ significantly from those not receiving interpleural analgesia in their previous studies. Additionally, they found that much of the instilled bupivacaine was lost in the drainage tubing. In a subsequent study of 20 patients [68], this group prevented loss of bupivacaine by clamping the drainage tubes, but found little advantage in comparison with i.m. oxycodone alone. Ferrante and colleagues [19] found that 30–40 % of administered bupivacaine was lost via the drainage tube, resulting in inadequate analgesia after thoracotomy.

It is well recognized that regional analgesia techniques may not be completely effective after thoracotomy and some post-thoracotomy pain has been attributed to other causes such as diaphragmatic irritation and scapular retraction [12]. This has been found for extradural analgesia and may also explain the inadequacy of interpleural analgesia in these patients.

Pain relief after renal surgery

Interpleural analgesia has been used successfully for percutaneous nephrostomy and nephrolithotomy, for extracorporeal shock wave lithotripsy (ESWL) and for pain management after open renal surgery. Trivedi, Robalino and Shevde [85] described four patients in whom interpleural analgesia (0.5 % bupivacaine 30 ml) was used without the need of supplementary sedative or analgesic drugs for nephrostomy and subsequent nephrolithotomy. Stromskag and Steen [82] reported the use of interpleural analgesia for ESWL in comparison with extradural analgesia. By random sequence 10 patients each received interpleural analgesia (2 % lignocaine 20 ml) or lumbar extradural 1.3 % mepivacaine 22–25 ml. In addition, both groups received fentanyl and diazepam during operation, as required. Pain was significantly greater in patients receiving interpleural analgesia at 15 and 30 min after the start of ESWL, but not at completion or 2 h later. Patients receiving interpleural analgesia required significantly more fentanyl than the extradural group.

Murphy [45] used interpleural analgesia after nephrectomy, but found that it was inadequate in four of the eight patients studied. It was felt that the

abdominal surgery. Schlesinger and colleagues [69] reported the use of interpleural analgesia as the sole anaesthetic technique for mammography during needle localization and for subsequent breast biopsy. Analgesia using this technique was adequate for surgery and no supplementary analgesia was required. Pain in the non-anaesthetized contralateral breast during its compression from mammography was greater in each case. Reiestad and McIlvaine [55] have also reported unilateral block of intercostal nerves (1st to 9th thoracic nerves), with complete skin anaesthesia sufficient for surgery to the breast. They recommended placing the patient in the lateral decubitus position with the affected side down and with a head-down tilt of 20° for 30 min after injection of the local anaesthetic.

Baxter and colleagues [6] compared interpleural analgesia in patients after cardiac surgery with a similar patient group receiving i.v. opioids. In these patients, interpleural catheters were inserted bilaterally and 0.25% bupivacaine 20 ml was instilled into each catheter every 6 h as required. Patients receiving interpleural analgesia recorded significantly smaller pain scores throughout the study, although the duration of analgesia was quite variable (requirement for injections ranged from one to four per day). Bilateral interpleural analgesia has also been described for pain relief after midline upper abdominal surgery [17, 35].

Pain relief for multiple fractured ribs

Interpleural analgesia is extremely effective in patients with multiple unilateral fractured ribs [25, 45, 49, 61]. The first use of interpleural analgesia was in a patient with five unilateral fractured ribs who achieved 8 h of pain relief from the first bolus of 0.5% plain bupivacaine 20 ml [49]. Subsequent injections gave relief of similar duration. Murphy [45] used this technique in 16 patients and found the duration of action was 8–12 h, with some patients requiring "top-ups" as infrequently as 18–24 hourly. Rocco and colleagues [61] achieved complete pain relief in nine patients with four to eight unilateral rib fractures, using either regular top-ups or a continuous infusion of bupivacaine.

Other indications for interpleural analgesia

Interpleural analgesia has been used in several uncommon situations, including management of cancer pain [15, 16, 20, 58], symptomatic management of acute herpes zoster [27] and in post-herpetic neuralgia [56, 58, 74, 75], pain management in chronic pancreatitis [1, 58, 75], reflex sympathetic dystrophy [57] and in the treatment of upper limb ischaemia [52]. In addition, interpleural analgesia has been advocated in situations in which other forms of pain management have been considered less desirable, such as in patients with cystic fibrosis [9] and in a patient with multiple rib fractures and a serious head injury [25].

Comparison of interpleural analgesia with other methods of pain relief

There have been few controlled studies comparing interpleural analgesia with other forms of analgesia.

Kastrissios and colleagues [29] compared interpleural analgesia with an i.v. infusion of pethidine in patients after cholecystectomy. Patients receiving interpleural analgesia received 0.5% bupivacaine 20 ml with adrenaline 1:200 000, followed by a continuous infusion of 0.25% bupivacaine 8 ml h⁻¹ without adrenaline and an additional 0.5% bupivacaine 20 ml with adrenaline after 5 h. The second group received pethidine 20–30 mg h⁻¹. Interpleural analgesia was associated with fewer side effects and with earlier mobilization, although the pain scores were similar for the two groups. Baxter and colleagues [6] used bilateral interpleural analgesia infusions in patients after cardiac surgery and found consistently smaller pain scores compared with patients receiving i.v. opioid. In contrast, Scheinin, Lindgren and Rosenberg [68] found that interpleural analgesia in patients after thoracotomy was no better than i.m. oxycodone.

Comparing interpleural local anaesthesia with interpleural saline after cholecystectomy in patients also receiving PCA morphine, Lee and colleagues [34] found a significant reduction in opioid requirement in the patients receiving local anaesthesia. In similar studies, VadeBoncouer and colleagues [87] and Mozell and colleagues [43] achieved similar results. In comparison with extradural local anaesthetic for pain relief after cholecystectomy, Scott and colleagues [70] found that extradural local anaesthesia was similar to interpleural analgesia for pain relief, pulmonary function and stress response to surgery. Stenseth and colleagues [77] found that extradural local anaesthesia was better than interpleural analgesia in a similar group. Stromskag and Steen [82] found that extradural lignocaine provided better operating conditions than interpleural analgesia for ESWL. Results obtained by Van Kleef, Burm and Vletter [88], who compared interpleural analgesia with multiple intercostal blocks in patients after gallbladder or renal surgery, showed that interpleural analgesia was of shorter duration, less reliable and associated with greater plasma concentrations of bupivacaine for a similar total dose.

Complications of interpleural analgesia

Complications of interpleural analgesia have been reviewed by Stromskag, Minor and Steen [79]. A total of 703 cases were included from 57 original articles and published abstracts. The most commonly registered complication was pneumothorax. This was documented in 14 patients. However, the authors cautioned against attempts to deduce the possible frequency of pneumothorax as, in many studies, chest x-ray findings were not reported, which makes interpretation impossible.

Evidence of unilateral sympathetic block after interpleural analgesia has been reported frequently [8, 38, 41, 51, 57, 74]. In most cases this was evident as a Horner's syndrome, and resolved without treatment. This unilateral sympathetic block has been used therapeutically by several authors [51, 57, 74]. Reiestad and colleagues [57] reported the use of interpleural analgesia in seven patients with reflex sympathetic dystrophy of the upper extremity. Using 0.5% bupivacaine 30 ml with

adrenaline, these authors reliably induced a profound sympathetic block by positioning the patient in a 20° head-down, lateral decubitus position with the affected side uppermost. Sympathetic block was confirmed by a Horner's syndrome, increase in skin temperature of the affected side and reduced sweating (cobalt blue filter paper test). Sihota and Holmblad [74] used the same technique in the management of post-herpetic neuralgia.

Isolated case reports have been published of other complications, including massive chest wall haematoma in a patient after cardiac surgery (using a modified technique for interpleural analgesia) [5], pleural effusion (which did not require surgical drainage) [47], catheter misplacement [24] and seizure activity in a patient under general anaesthesia [72]. Increased plasma concentrations and systemic toxicity from local anaesthesia have also been reported (see below).

PHARMACOLOGY

Plasma concentrations of local anaesthetic drugs after interpleural analgesia

There is great difficulty in interpreting the significance of plasma concentrations of local anaesthetic drugs after regional anaesthesia [86]. For this reason, many authors have measured plasma concentrations of lignocaine and bupivacaine after interpleural analgesia without any attempt at correlation with clinical response [2, 10, 23, 31, 36, 38, 40, 63, 66, 67, 72, 81, 88]. The estimated threshold plasma concentrations associated with the onset of CNS toxicity for lignocaine and bupivacaine are usually quoted at 5–10 µg ml⁻¹ and 2–4 µg ml⁻¹, respectively [86]. It may be, however, that the rate of change in plasma concentration is more important in determining clinical toxicity than the absolute blood concentrations [50]. For this reason, most authors evaluating interpleural analgesia have concentrated on determination of the peak plasma concentration (*C*_{max}) and the time to reach this concentration (*t*_{Cmax}). Many authors measured plasma concentrations in venous blood. Mogg and colleagues [40] compared arterial with venous plasma concentrations of bupivacaine after interpleural analgesia and showed that a 20% variation occurred between the two routes. *C*_{max} for arterial samples was 1.9 (SD 0.36) µg ml⁻¹ and was attained at 22 (10) min. Venous *C*_{max} was 1.65 (+0.48) µg ml⁻¹ at 25 (10) min. Van Kleef, Burm and Vletter [88] observed a difference of 10% variation between arterial and venous samples.

As might be expected, *C*_{max} increased with increasing dose of local anaesthetic. Stromskag and colleagues [81] compared arterial plasma concentrations in three groups of 10 patients receiving 0.25%, 0.375% or 0.5% bupivacaine 20 ml with adrenaline. *C*_{max} was 0.62 (0.25) µg ml⁻¹, 0.82 (0.4) µg ml⁻¹ and 1.2 (0.44) µg ml⁻¹, respectively, all peak concentrations occurring at approximately 15 min after injection. The influence of volume of a given dose has been studied for lignocaine by Kuhlman and colleagues [31]. These authors administered 2% or 0.5% lignocaine 2 mg kg⁻¹ with

adrenaline. Venous blood samples reached *C*_{max} 0.97 (0.46) µg ml⁻¹ and 0.52 (0.28) µg ml⁻¹; *t*_{Cmax} was 34 (13) min and 48 (14) min for the 2% and 0.5% concentrations, respectively.

Most authors used local anaesthetic with adrenaline, with the expectation of reducing the rate of drug absorption. Gin and colleagues [23] examined the effect of addition of adrenaline to the bupivacaine solution and confirmed that the peak plasma concentration was smaller (venous *C*_{max} 2.57 µg ml⁻¹ compared with 3.22 µg ml⁻¹) and the time to reach this peak, longer (*t*_{Cmax} 25 min compared with 15 min) with addition of adrenaline. There was no difference in total body clearance, apparent volume of distribution or elimination half-life between the two groups. Rademaker and colleagues [53] showed no such difference associated with addition of adrenaline.

After the commonly used dose of 0.5% bupivacaine 20 ml with adrenaline, the maximum arterial concentration of bupivacaine may be expected to be approximately 2 µg ml⁻¹ within 15–20 min of injection and may be expected to increase with administration of repeated doses or with use of a continuous infusion [63, 67]. Despite the large concentrations attained in some patients, clinical toxicity is rarely described in these patients. Only one instance of seizure activity has been reported [72]. This occurred when the first dose of bupivacaine was injected to a patient after cholecystectomy. The extradural catheter was inserted at termination of surgery. Five millilitre of straw-coloured fluid was aspirated through the catheter, but as no further fluid could be obtained, bupivacaine was injected. Within 1 min of the bupivacaine injection (0.5% bupivacaine 30 ml with 1:100 000 adrenaline over 2-min), seizure activity was noted in the head and neck region of the patient, who remained under general anaesthesia. Cardiovascular depression did not occur, but awakening was noted to be prolonged. A maximum venous concentration of bupivacaine 4.9 µg ml⁻¹ was found 5 min after injection of bupivacaine. The authors felt that the pleura may have been inflamed after a recent pneumonia and contributed to rapid systemic absorption.

Effect on pulmonary function

Few authors have attempted to determine the effect of interpleural analgesia on postoperative pulmonary function. Murphy [44], using intermittent doses of bupivacaine after cholecystectomy, measured peak expiratory flow (using a Wrights spirometer) on the first day after operation when the patients requested analgesia. Thereafter, the interpleural bupivacaine was administered and peak flow remeasured 30–40 min later. Peak flow after top-up (212 (19) litre min⁻¹) was significantly greater than that before top-up (154 (14) litre min⁻¹) (*P* < 0.01).

VadeBoncouer and colleagues [87] measured FEV₁ and FVC on the first day after operation before and 1 h after an interpleural injection of bupivacaine or saline. FEV₁ and FVC in patients receiving bupivacaine were significantly greater 1 h after administration of bupivacaine. Patients receiving saline showed no significant change in these measurements.

Chan and colleagues [11] achieved similar results comparing 6-hourly bolus doses of bupivacaine or saline. In contrast, Oxorn and Whatley [50] found that i.m. pethidine was better than interpleural analgesia with regard to pulmonary function.

CONCLUSIONS

Interpleural analgesia is clearly effective in patients after open cholecystectomy and in patients with multiple unilateral fractured ribs. In these situations, interpleural analgesia consistently provides high quality analgesia with few adverse effects and may be recommended as a safe alternative to thoracic extradural analgesia. The same consistency of effect has not been found for interpleural analgesia after thoracotomy. It is possible that variations in the technique used may be responsible for the disparity in results. Some authors attempt to place the extradural catheter in the tissue plane superficial to the parietal pleura, close to or in the paravertebral space. In this situation, perhaps less local anaesthetic is lost through thoracic drainage tubes and contributes to a more effective block. Interpleural analgesia has been used in other painful conditions for acute and chronic pain management with good effect, but these indications are relatively rare. Insufficient data are available to allow adequate comparison with other forms of analgesia.

Systemic absorption of bupivacaine from the pleural cavity may be sufficient to produce large plasma concentrations of bupivacaine, especially if given as a continuous infusion over 2 days or more. The correlation between plasma concentration and clinical effect is poor, however, and little useful information can be derived from plasma bupivacaine sampling. Clinical signs of toxicity have rarely been reported for interpleural analgesia.

Several variations on the technique of interpleural analgesia are described, but there appears to be little to choose between them. The effect in each case is thought to be the result of neuronal block at or lateral to the paravertebral space. There is a possibility of some extradural spread. Adverse effects are uncommon; in particular, pneumothorax does not appear to be a significant problem, but the true incidence of this complication is unknown.

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SHORT COMMUNICATIONS

PATIENT-CONTROLLED ANALGESIA WITH A MIXTURE OF MORPHINE AND DROPERIDOL

S. K. SHARMA AND M. W. DAVIES

SUMMARY

In a prospective study, 50 women who had undergone uncomplicated abdominal hysterectomy under general anaesthesia were allocated randomly to receive patient-controlled analgesia with either morphine alone or a mixture of morphine and droperidol. Bolus doses of morphine 1 mg and droperidol 0.05 mg were used with a lockout time of 5 min. During the first 24 h after surgery the mean (range) dose of droperidol in the droperidol group was 3.2 (1.9–6.0) mg. Significantly fewer patients in the droperidol group felt nauseated ($P < 0.01$) and significantly fewer vomited ($P < 0.001$). In the morphine alone group, 19 patients required additional antiemetic therapy, whereas in the droperidol group only one patient found this necessary ($P < 0.001$). Extrapyramidal side effects were not observed in any patient. Significantly more patients were of the opinion that PCA had provided excellent analgesia when droperidol had been used ($P < 0.01$). (Br. J. Anaesth. 1993; 71: 435–436)

KEY WORDS

Analgesia: patient controlled. Antiemetics: droperidol. Complications: nausea, vomiting.

Patient-controlled analgesia (PCA) provides good postoperative analgesia [1], but is associated with a high incidence of postoperative nausea and vomiting (PONV) [2, 3]. Low-dose droperidol given during anaesthesia has been shown to reduce PONV during the early postoperative period [4]. For some time, we have used a mixture of morphine and droperidol for PCA and this seems to have reduced greatly the incidence of both nausea and vomiting. This study was designed to compare the incidence of PONV with PCA using either morphine alone or a mixture of morphine and droperidol.

METHODS AND RESULTS

The District Pharmacy confirmed that droperidol and morphine could be mixed with no evidence of physical incompatibility. Ethics Committee approval and written informed consent were obtained. We studied 50 patients (ASA I and II) who had undergone uncomplicated abdominal hysterectomy under general anaesthesia. Patients were premedicated with temazepam 20 mg 2 h before surgery. Those receiving other medication before operation

were excluded. Anaesthesia was induced with fentanyl $2 \mu\text{g kg}^{-1}$, droperidol $20 \mu\text{g kg}^{-1}$ and propofol $1.5\text{--}2.5 \text{ mg kg}^{-1}$ and was maintained with isoflurane and 65% nitrous oxide in oxygen. Neuromuscular block was produced with atracurium 0.5 mg kg^{-1} . Ventilation was adjusted to maintain the end-tidal partial pressure of carbon dioxide at $4.7\text{--}5.3 \text{ kPa}$. Neostigmine $40 \mu\text{g kg}^{-1}$ and atropine $20 \mu\text{g kg}^{-1}$ were given to antagonize residual neuromuscular block at the end of the procedure. PCA (Abbott) was started immediately after recovery from anaesthesia. Using consecutive sealed envelopes, patients were allocated randomly to receive either morphine alone (bolus dose 1 mg) or a mixture of morphine (bolus dose 1 mg) and droperidol (bolus dose 0.05 mg). A background infusion was not given and the lockout time for both groups was 5 min. Prochlorperazine 12.5 mg 6 hourly, as required, was prescribed for relief of PONV.

Assessments of PONV and morphine and prochlorperazine usage were made by nursing staff for six consecutive, 4-h periods following surgery. Nursing staff were unaware of the study group; PONV was graded as: no nausea, nausea alone, vomiting or retching. All patients were examined by one of the authors 24 h after operation for evidence of excessive sedation or extrapyramidal side effects (restlessness, abnormal movements, tremor). Quality of analgesia during the 24 h was graded by the patient as: awful, poor, satisfactory, good or excellent.

Groups were compared using Student's t test and chi-square test, with Yates's correction; $P < 0.05$ was regarded as significant.

Four patients in each group required prochlorperazine during the first 1 h after surgery. These data were excluded from the main analysis as it was felt that PONV during the immediate postoperative period was more likely to be related to anaesthesia and surgery rather than to PCA. Patient data and morphine requirements were similar for both groups (table I). Patients in the morphine alone group were significantly more likely to feel nauseated, to vomit and to require prochlorperazine than those in the droperidol group (table I). Of the eight patients who

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TABLE I. Patient data (mean (range)), postoperative nausea and vomiting, and requirement for prochlorperazine (excluding first 1 h after operation). ** $P < 0.01$; *** $P < 0.001$ between groups

	Morphine only (n = 21)		Morphine + droperidol (n = 21)
Weight (kg)	61.3 (52–70)		58.4 (52–68)
Age (yr)	43.0 (30–59)		43.8 (28–65)
Total morphine (mg)	62.3 (34–127)		63.5 (38–120)
Total droperidol (mg)	—		3.2 (1.9–6.0)
Nauseated (No.)	20	**	10
Vomited (No.)	11	***	0
Prochlorperazine (No.)	19	***	1

TABLE II. Satisfaction with analgesia during first 24 h after operation. ** $P < 0.01$ between groups

	Morphine (n = 21)		Morphine + droperidol (n = 21)
Awful	0		0
Poor	1		1
Satisfactory	3		4
Good	17		7
Excellent	0	**	9

required prochlorperazine during the first 1 h after operation, none in the droperidol group requested a subsequent dose, whereas all those in the morphine alone group needed additional doses.

Significantly more patients were of the opinion that PCA had provided excellent analgesia when droperidol had been used (table II). Excessive sedation or extrapyramidal side effects were not observed in any patient.

COMMENT

As with other studies [2, 3], we found a very high incidence of PONV when morphine alone was used for PCA. The doses of droperidol administered were small, but were nonetheless effective in reducing both nausea and vomiting. Vomiting was almost abolished and the incidence of nausea was halved.

Including the bolus dose given at induction of anaesthesia, patients received, on average, approximately 3.2 mg of droperidol during the 24-h period. Had the droperidol been given as a single dose at induction of anaesthesia, the effective duration of action would have been less and side effects may have been troublesome [5].

Sedation is a well recognized effect of droperidol, but did not seem to be a problem with the doses used. All patients were either awake or easily rousable at 24 h. This technique appears to control PONV associated with PCA more effectively and with fewer side effects than transdermal hyosine [6]. The concept of mixing morphine with an antiemetic is well established; a mixture of morphine and cyclizine (Cyclimorph, Calmic.) has been available commercially for many years. The mixture of morphine and droperidol appeared to be stable, as both drugs remained clinically effective and no precipitate was visible after 24 h. One advantage of PCA is that fewer i.m. injections need be given during the postoperative period. The addition of low-dose droperidol to the morphine used for PCA further reduces the need for injections and, moreover, may enhance the quality of analgesia as perceived by the patient.

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PHARMACOKINETICS OF GLYCOPYRRONIUM IN URAEMIC PATIENTS

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SUMMARY

We studied the pharmacokinetics of glycopyrronium in 11 uraemic patients undergoing cadaveric renal transplantation and in seven ASA I control patients undergoing general surgery. Glycopyrronium $4 \mu\text{g kg}^{-1}$ was given i.v. before induction of anaesthesia. Blood and urine samples were collected for up to 24 h for measurement of glycopyrronium concentrations using a radioreceptor assay. Volume of distribution in the elimination phase (V^{β}) was similar in both groups, the elimination half-life ($T_{1/2}^{\beta}$) was longer ($P < 0.05$), area under the plasma concentration-time curve (AUC) larger ($P < 0.01$) and plasma clearance (Cl) smaller ($P < 0.01$) in the uraemic patients. In 3 h, mean 0.7 (range 0-3) % and 50 (21-82) % of glycopyrronium was excreted in the urine in the uraemic and healthy patients, respectively ($P < 0.001$). The 24-h renal excretion was 7 (0-25) % in uraemic and 65 (30-99) % in control patients ($P < 0.001$). We conclude that the elimination of glycopyrronium is severely impaired in uraemic patients. (Br. J. Anaesth. 1993; 71: 437-439)

KEY WORDS

Kidney: failure. Pharmacokinetics: glycopyrronium.

Eighty per cent of glycopyrronium is excreted unchanged in the urine [1]. The metabolic pathways of the remaining 20 % are unknown, and little is known about the pharmacokinetics of glycopyrronium in patients with renal failure.

The development of a sensitive radioreceptor assay (RRA) has enabled us to measure concentrations as small as 60 ng litre^{-1} and thus to determine the plasma and urinary concentrations of the drug [2]. We have studied the pharmacokinetics of glycopyrronium in uraemic patients undergoing renal transplantation.

METHODS AND RESULTS

We studied 11 uraemic patients undergoing renal transplantation and seven ASA I control patients undergoing general surgery. Patients receiving medications which could interfere with the RRA were excluded. The study was approved by the Hospital Ethics Committee and all patients gave informed consent.

Mean serum creatinine concentration was $731 (\text{SD } 267) \mu\text{mol litre}^{-1}$ and $75 (17) \mu\text{mol litre}^{-1}$ in the uraemic and control patients, respectively. The serum albumin concentrations in the uraemic patients were normal. The patients had no known liver dysfunction, and in the uraemic patients serum concentrations of transaminases and prothrombin time were within normal limits. The uraemic and control patients were comparable in age and weight.

All the uraemic patients had undergone renal dialysis within the previous 24 h. Patients were premedicated with oral diazepam 0.2 mg kg^{-1} . In the operating theatre, a forearm vein was cannulated and in the uraemic patients a central venous catheter was inserted. Thereafter, potassium-free Ringer's acetate ($\text{Na}^+ 140$, $\text{Ca}^{2+} 2$, $\text{Cl}^- 90$, $\text{CH}_3\text{COO}^- 54 \text{ mmol litre}^{-1}$) and 4 % human albumin solution were administered until central venous pressure was at least 4 mm Hg. Control patients received an infusion of Ringer's acetate solution (approximately 500 ml) as volume loading. Glycopyrronium $4 \mu\text{g kg}^{-1}$ was given i.v. via the peripheral cannula. Approximately 15 min after injection of glycopyrronium, patients were anaesthetized with thiopentone $3-5 \text{ mg kg}^{-1}$ and fentanyl $0.15-0.25 \text{ mg i.v.}$ Vecuronium $1 \text{ mg} + 0.1 \text{ mg kg}^{-1}$ was used to produce neuromuscular block. Anaesthesia was maintained with fentanyl and isoflurane with 70 % nitrous oxide in oxygen. Blood samples (10 ml) were taken into EDTA tubes before and 2, 4, 6, 10, 15 and 30 min and 1, 2, 3, 6, 12 and 24 h after injection of glycopyrronium. Samples were obtained from the central venous catheter in the uraemic patients and from a peripheral cannula inserted into the contralateral cubital vein in control patients. Plasma was separated and stored at -70°C . Urine samples were collected 0-3, 3-6, 6-8, 8-12 and 12-24 h after the injection of glycopyrronium. Urine volumes were measured and samples taken from each fraction for measurement of glycopyrronium.

The concentrations of glycopyrronium in plasma and urine were measured using the radioreceptor assay (RRA) described by Kaila and colleagues [2].

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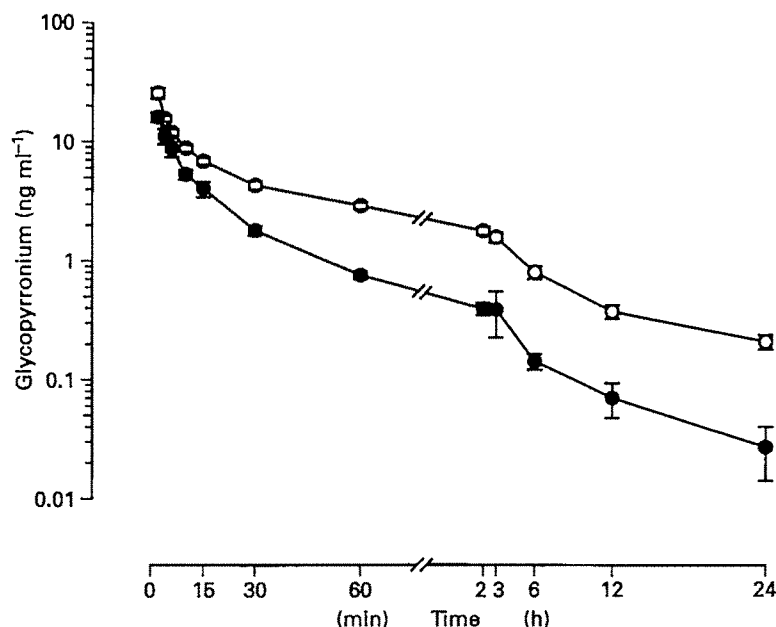


FIG. 1. Mean (SEM) plasma concentrations of glycopyrronium in 11 uraemic (○) and seven control (●) patients.

The sensitivity of the assay for glycopyrronium was 60 pg ml⁻¹ and the coefficients of intra-assay and interassay variation were less than 10%. The pharmacokinetic parameters were calculated by fitting the concentration-time data to a bi-exponential model:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t},$$

where C = plasma concentration of glycopyrronium at time t ; A, B = zero-time intercepts; α, β = rate constants for disposition and elimination.

For computations, a commercial software package was used (PCNonlin 3.0 SCI Software, Lexington, KY, U.S.A.). AUC values were calculated using the trapezoidal rule.

If serum creatinine decreased significantly within the first day after operation, this was considered as immediate kidney function.

Pharmacokinetic data were analysed with the Mann-Whitney U test, using Stat View 512+ software (Brain Power Inc., Calabasas, CA, U.S.A.).

One of the 11 transplanted kidneys showed immediate function. The serum creatinine concentration in this patient was 1123 $\mu\text{mol litre}^{-1}$ before operation and 699 $\mu\text{mol litre}^{-1}$ on the first day after operation. In the remainder of the 10 uraemic patients, serum creatinine concentration remained at preoperative values during the first day after operative day.

Figure 1 shows the mean plasma concentrations of glycopyrronium in both groups. Volume of distribution in the elimination phase (V^{β}) was 0.41 (0.16) litre kg⁻¹ and 0.44 (0.33) litre kg⁻¹, elimination half-life ($T_{1/2}^{\beta}$) was 0.78 (0.45) h and 0.31 (0.29) h ($P < 0.05$), the area under the concentration-time curve (AUC) 10.62 (3.29) h $\mu\text{g litre}^{-1}$ and 3.73 (1.03) h $\mu\text{g litre}^{-1}$ ($P < 0.01$) and the plasma clearance (Cl) 0.43 (0.22) litre h⁻¹ kg⁻¹ and 1.14 (0.31) litre h⁻¹ kg⁻¹ ($P < 0.01$) in the uraemic and healthy patients, respectively. In 3 h, a mean 0.7 (range 0–3)% and 50 (21–82)% of glyco-

pyrronium was excreted in the urine in the uraemic and healthy patients, respectively ($P < 0.001$). The 24-h renal excretion was 7 (0–25)% in uraemic and 65 (30–99)% in control patients ($P < 0.001$). In the patient with immediate postoperative kidney function, 25% of glycopyrronium was excreted in the urine in 24 h, but the plasma concentrations of glycopyrronium in this patient did not differ from those in the other uraemic patients. Probably, the plasma concentrations of glycopyrronium in this patient would have been even greater without this increased excretion rate. The interindividual variation of plasma glycopyrronium concentrations was large; therefore no conclusions can be drawn from this one subject.

COMMENT

These data indicate that the elimination of glycopyrronium in plasma was significantly prolonged in uraemic patients compared with non-uraemic control patients. Theoretically, using the central venous catheter in the uraemic patients and a peripheral venous cannula in the control patients for sampling might have affected the results. However, because venous blood was sampled in both groups and a large cubital vein with good flow was used in the control patients, it is very unlikely that this would have changed our conclusions. The effects of anaesthetics, other medication or protein binding on the pharmacokinetics of glycopyrronium are unknown; our opinion is that they did not influence our results. The $T_{1/2}^{\beta}$ of glycopyrronium (0.78 h) in the uraemic patients was similar to that (0.83 h) in elderly patients (mean age 66 yr) with no renal disease reported in a previous study by Ali-Melkkilä, Kaila and Kanto [3]. This might be attributable in part to an approximately 50% greater V^{β} in elderly patients (0.64 litre kg⁻¹) compared with our uraemic group.

In uraemic patients, the average 24-h renal excretion of glycopyrronium was only 7%, whereas the control patients excreted 65% of the dose within

24 h. It has been shown previously that the 8-h excretion of glycopyrronium is approximately 60% after i.m. administration [4].

It should be noted that, 6 h after drug administration, plasma concentrations of glycopyrronium were nearly 10 times greater in our uraemic patients compared with the control patients, and that in the uraemic patients the concentration was 200–300 pg ml⁻¹ 24 h after i.v. administration. These small concentrations have little effect on heart rate, but may produce prolonged antisecretory effects [3].

Anticholinergic drugs also disrupt peristaltic bowel activity [5], and glycopyrronium decreases gastric emptying by 40–50%, when used in doses similar to those in our study [6]. Thus, in uraemic patients, delayed bowel function after surgery caused by opioids and other medication may be aggravated by the use of glycopyrronium.

We conclude that the elimination of glycopyrronium is severely impaired in uraemic patients. Therefore we feel that, in these patients, repeated or

large doses of glycopyrronium should be avoided or, perhaps, the drug should not be used.

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SYNERGISM BETWEEN ATRACURIUM AND VECURONIUM IN CHILDREN

O. A. MERETOJA, B. W. BRANDOM, T. TAIVAINEN AND L. JALKANEN

SUMMARY

In 30 children under balanced anaesthesia, we have determined dose-response curves and maintenance requirement of three dose ratio combinations of atracurium and vecuronium (10:1, 4:1 or 1.6:1 on a $\mu\text{g}:\mu\text{g}$ basis). Neuromuscular block was monitored by adductor pollicis EMG. An equipotent dose ratio (4:1) was most potent, with a mean (SEM) ED_{95} of atracurium $95 (6) \mu\text{g kg}^{-1}$ with vecuronium $24 (1) \mu\text{g kg}^{-1}$. The sum of these doses is only 58% of an ED_{95} value of one agent ($P = 0.0001$). The hourly requirement to maintain a 90–95% neuromuscular block was 2.0 (0.1) times an individual ED_{95} dose of any combination. Recovery index was 8.9 (0.5) min. These results indicate that a combination of atracurium and vecuronium is supra-additive compared with the effects of each drug alone. However, all combinations maintained an intermediate character of neuromuscular block. Combining atracurium with vecuronium may reduce drug requirement by 40%. (Br. J. Anaesth. 1993; 71: 440–442)

KEY WORDS

Neuromuscular relaxants: atracurium, vecuronium. Pharmacodynamics: relaxant combination.

Synergism (supra-additivity) of the neuromuscular blocking effects between some competitive neuromuscular blocking agents of different molecular structure has been observed both *in vivo* and *in vitro* [1, 2]. There are also some data suggesting pure additive effects of this combination [3]. The subject is further confused because one study found non-parallel and another found parallel slopes of the dose-response curves between a combination of neuromuscular blocking agents and their parent agents [1, 2]. Our aim was to clarify the nature of the neuromuscular block produced by a combination of atracurium and vecuronium.

METHODS AND RESULTS

After obtaining Institutional Ethics Committee approval and parental informed consent, we studied 30 children (ASA I–II), aged 3–10 yr, undergoing routine general surgery, allocated randomly to three matched groups. Premedication comprised oral midazolam $0.46 (\text{SEM } 0.01) \text{ mg kg}^{-1}$. General anaesthesia was induced with thiopentone

$5.2 (0.2) \text{ mg kg}^{-1}$, alfentanil $38 (2) \mu\text{g kg}^{-1}$ and 66% nitrous oxide in oxygen, and maintained with nitrous oxide in oxygen and alfentanil. Neuromuscular block was monitored at 20-s intervals by the evoked adductor pollicis EMG response to supramaximal train-of-four ulnar nerve stimuli (Relaxograph, Datex). Palmar skin temperature was maintained at $> 34^\circ\text{C}$ and end-tidal concentrations of carbon dioxide at 5.0–5.5%.

Each patient received one of three dose ratio combinations of atracurium and vecuronium: an equipotent dose ratio of 4:1 (on a $\mu\text{g}:\mu\text{g}$ basis), or a ratio of 10:1 or 1.6:1 (these represent geometric mean ratios for an equipotent ratio and a parent agent) (fig. 1A). An individual cumulative dose-response curve of the combination was created. The first dose was atracurium $40 \mu\text{g kg}^{-1}$ and vecuronium $10 \mu\text{g kg}^{-1}$ in a 4:1 dose ratio group, atracurium $60 \mu\text{g kg}^{-1}$ and vecuronium $6 \mu\text{g kg}^{-1}$ in a 10:1 dose ratio group, and atracurium $24 \mu\text{g kg}^{-1}$ and vecuronium $15 \mu\text{g kg}^{-1}$ in a 1.6:1 dose ratio group. A second identical dose was administered when the maximal neuromuscular block after the first dose was reached. A third incremental dose was calculated to establish a 95% neuromuscular block. A least-square linear regression analysis of log doses and probit responses was used to determine an individual dose-response curve of each combination of atracurium and vecuronium.

During maintenance of anaesthesia, 25% of an individual ED_{95} dose of combination was administered each time the neuromuscular function recovered to 10% of baseline. Neuromuscular function recovered spontaneously at the end of anaesthesia, whenever clinically appropriate. Analysis of variance and Scheffé F test were used to evaluate between-group comparisons.

There was little deviation from the linear regression of log doses and probit responses ($r^2 = 0.995 (0.001)$). Slopes of the individual dose-response curves did not differ between groups ($P = 0.27$) and averaged 7.0 (0.1) probit/log. This did not differ from that of parent agents [4]. An ED_{50} and an ED_{95} value of an equipotent dose-ratio combination were 0.62 (0.04) and 0.58 (0.04) times

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dose ratio favouring one parent agent. These synergistic combinations may be used as one intermediate acting competitive neuromuscular blocking agent. Maximal reduction of drug requirement would be about 40 %.

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RELATIONSHIP BETWEEN POST-TETANIC TWITCH AND SINGLE TWITCH RESPONSE AFTER ADMINISTRATION OF VECURONIUM

Y. SAITOH, H. TOYOOKA AND K. AMAHA

SUMMARY

We have studied the relationship between post-tetanic twitch (PTT) and single twitch response after administration of vecuronium 0.2 mg kg^{-1} in 100 patients during neuroleptanaesthesia (NLA) (droperidol and fentanyl) and during anaesthesia with halothane, isoflurane, enflurane or sevoflurane (1 MAC in nitrous oxide and oxygen). Intervals from PTT, to single twitch, and post-tetanic-count (PTC) (number of PTT responses) at the onset of single twitch were determined electromyographically. These intervals in the isoflurane, enflurane and sevoflurane groups differed from those in NLA and halothane groups. PTC in the isoflurane, enflurane and sevoflurane groups differed from those in the NLA group, and PTC in the sevoflurane group significantly from those in the NLA and halothane groups. These results are consistent with the view that PTT reflects prejunctional block, whilst the single twitch response is indicative of postjunctional block. (Br. J. Anaesth. 1993; 71: 443-444)

KEY WORDS

Monitoring: post-tetanic twitch. Neuromuscular relaxants: vecuronium.

Absence of post-tetanic twitch (PTT) indicates intense neuromuscular block, because a PTT response appears earlier than single twitch or train-of-four (TOF) responses during the recovery of neuromuscular transmission [1]. We have reported previously the time courses of recoveries of PTT and TOF after administration of vecuronium under different types of anaesthesia [2]. In this study, we have investigated the relationship between PTT and single twitch during neuroleptanaesthesia (NLA) and during anaesthesia with halothane, isoflurane, enflurane and sevoflurane.

METHODS AND RESULTS

We studied 100 adult patients (54 male), ASA I or II, undergoing elective general surgical, orthopaedic, gynaecological, urological, ENT and ophthalmological procedures, after obtaining institutional Review Board approval and written informed consent. None of the patients had neuromuscular, renal or

hepatic disorders or was receiving any medications that might affect neuromuscular transmission. The patients were allocated randomly to five groups (20 patients in each) to receive: neuroleptanaesthesia (NLA group), halothane, isoflurane, enflurane or sevoflurane. All patients were premedicated with atropine 0.01 mg kg^{-1} and hydroxyzine 1 mg kg^{-1} i.m.

Stimulating surface electrodes were placed on the ulnar nerve at the elbow and recording electrodes on the abductor digiti minimi muscle. Anaesthesia was induced with sodium thiamylal 5 mg kg^{-1} , then the ulnar nerve was stimulated supramaximally (50 mA) with an electrical stimulator (SEN-3201, Nihon-Kohden Inc., Tokyo). Rectangular pulses of 0.1 ms duration were triggered automatically by computer as described elsewhere [2]. When the response to single twitch stimuli was stable, the magnitude of the single twitch was taken as the control twitch height.

Vecuronium 0.2 mg kg^{-1} i.v. was administered to facilitate tracheal intubation. Anaesthesia was maintained with 66% nitrous oxide in oxygen and fentanyl $5 \mu\text{g kg}^{-1}$ and droperidol 0.2 mg kg^{-1} in the NLA group, and with 66% nitrous oxide in oxygen and 1 MAC of inhalation anaesthetics (end-tidal) in the four other groups (0.76% halothane, 1.15% isoflurane, 1.68% enflurane or 1.17% sevoflurane). End-tidal concentrations of carbon dioxide, nitrous oxide and inhalation anaesthetics were measured using a Capnomac Ultima-S-31-03 (Datex Inc., Helsinki). The patient's lungs were ventilated to normocapnia. Body temperature was maintained at $35.5\text{--}36.8^\circ\text{C}$ using a warming blanket.

For PTT stimulation, a tetanic stimulus (50 Hz) was applied for 5 s and after an interval of 3 s, single twitch stimuli (1 Hz) were applied for 20 s. The twitch height of each stimulus was amplified via an amplifier (AVB-11, Nihon-Kohden Inc., Tokyo) and displayed on an oscilloscope (VC-11, Nihon-Kohden Inc., Tokyo).

Single twitch stimuli were given every 30 s and PTT stimuli every 150 s. Thereafter, we measured the intervals from PTT₁ to single twitch and PTC (number of PTT responses) at the onset of single

TABLE I. Intervals from PTT₁ to single twitch (mean (SD)) and PTC at the onset of single twitch (mean (range)). NLA = Neuroleptanaesthesia. Significant difference ($P < 0.01$) compared with **NLA; group; †† halothane

	NLA	Halothane	Isoflurane	Enflurane	Sevoflurane
PTT ₁ -single twitch interval (min)	8.0 (3.3)	9.1 (3.5)	14.4 (3.3)**††	14.8 (4.0)**††	15.3 (4.5)**††
PTC at the onset of single twitch	7.3 (4-10)	7.9 (6-11)	8.9 (6-14)**	9.3 (7-14)**	10.1 (7-16)**††

twitch during recovery of neuromuscular transmission. The maximum PTC which had been detected before the first single twitch response appeared was designated the "PTC at the onset of single twitch".

The responses to electrical stimulation were considered detectable when the changes were larger than or equal to 1% of control twitch height.

Data were analysed statistically using ANOVA and Duncan's multiple range test [3], $P < 0.01$ being considered significant. All results are expressed as mean (SD or range).

There were no significant differences in patient characteristics (age, sex, height and weight) between groups.

Intervals between PTT₁ and single twitch in the isoflurane, enflurane and sevoflurane groups differed significantly from those in the NLA and halothane groups. PTC at the onset of single twitch in the isoflurane, enflurane and sevoflurane groups differed from those in NLA group, and those in the sevoflurane group differed significantly from those in the NLA and halothane groups (table I).

COMMENT

We have found that the intervals from PTT₁ to single twitch, and PTC at the onset of single twitch differed significantly between the different anaesthetic groups. These findings suggest that the relationship between PTT and single twitch varies with different types of anaesthesia.

We reported previously that, during recovery of vecuronium-induced neuromuscular transmission, the appearance of PTT followed a similar time course with five types of anaesthesia (neuroleptanaesthesia, halothane, isoflurane, enflurane or sevoflurane), while those of T1, T2, T3 and T4 were delayed to various extents [2].

PTT is thought to indicate a prejunctional effect of non-depolarizing neuromuscular blocking agent. In contrast, the single twitch or TOF twitch height is thought to reflect postjunctional effects [2]. Inhalation anaesthetics prolong neuromuscular block by acting mainly on the postjunctional region of the neuromuscular junction [2], thus delaying the onset of the single twitch response, but not the PTT. The prolongation of PTT₁-single twitch intervals in

the isoflurane, enflurane and sevoflurane groups would result entirely from the delayed onset of the single twitch. Similarly, the finding that PTC at the onset of single twitch varied according to the anaesthetic used results from the difference in time to the onset of single twitch in the five groups.

The concentrations of isoflurane and enflurane at the neuromuscular junction increase slowly and reach equilibrium after 90 min [4] and 200-240 min [5], respectively. However, the PTT₁ and single twitch appeared after approximately 30-45 min from induction of anaesthesia in our study, so the concentration of inhalation anaesthetic at the neuromuscular junction would have been small and should have had little influence on our data. In addition, the time-dependent increase is slight [4,5].

PTT stimuli were carried out at 2.5-min intervals in our study. This relatively short interval was used for more precise determination of the relationship between PTT and single twitch. However, this may have affected spontaneous recovery of neuromuscular block in the investigated arm [1,6]. Ali [6] reported that tetanic stimulation may increase muscle blood flow, and this would promote delivery of neuromuscular blocking agent to the neuromuscular junction and intensify the block. This suggestion requires further investigation.

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EFFECT OF AGE ON EXTRADURAL DOSE REQUIREMENT IN THORACIC EXTRADURAL ANAESTHESIA

Y. HIRABAYASHI AND R. SHIMIZU

SUMMARY

We have investigated the effect of age on thoracic extradural dose requirements in 62 patients aged 20-79 yr. Fifteen millilitre of 2% mepivacaine was administered at a constant rate (1 ml s^{-1}) by syringe pump through an extradural needle at the T9-10 interspace. A mean of 15.5 (SD 4.2) spinal segments were blocked 15 min after completion of the administration. The extradural dose requirement decreased with increasing age ($r = -0.7$, $P < 0.001$), the requirement in the elderly (60-79 yr) being about 40% smaller than that in young adults (20-39 yr). (Br. J. Anaesth. 1993; 71: 445-446)

KEY WORDS

Age factors: extradural anaesthesia. Anaesthetic techniques: extradural. Anaesthetics, local: mepivacaine.

Although many investigators have reported the effect of ageing on the extradural dose requirement in lumbar extradural anaesthesia, few have examined the effect of ageing on the extradural dose requirement in thoracic extradural anaesthesia [1]. Some authors have reported a reduction in lumbar extradural dose requirements with age [2, 3], while others have demonstrated that age has little or no effect on the spread of lumbar extradural anaesthesia [4].

If the dose requirement in thoracic extradural anaesthesia does vary with age, to what degree it varies remains unknown. We therefore investigated the effect of ageing on dose requirements in thoracic extradural anaesthesia.

METHODS AND RESULTS

We studied 62 patients aged 20-79 yr who required extradural anaesthesia for upper abdominal surgery. All gave informed consent to the study, which was approved by the Hospital Ethics Committee. All patients were premedicated with atropine 0.5 mg and hydroxyzine 25-50 mg i.m. 1 h before surgery.

The patient was placed in the right lateral position on a horizontal operating table. A 17-gauge Tuohy needle, the bevel of which was pointing cephalad, was introduced at the T9-10 interspace. The interspace was identified by examination and palpation of the spine, counting upward from the line joining the iliac crests which was assumed to correspond with L3-4.

The extradural space was identified using the dripping infusion method. When no cerebrospinal fluid or blood flowed out of the needle, 2% mepivacaine 15 ml was injected into the extradural space through the needle at a constant rate of 1 ml s^{-1} by a syringe pump (Kimura Medical, Japan). An extradural catheter was inserted to 5 cm beyond the bevel point of the needle and the patient was turned to the supine position. All blocks were performed by the same anaesthetist. Fifteen minutes after the injection, the upper and lower border of analgesia to pinprick according to a dermatome chart was assessed on the left and right sides. The number of spinal segments blocked was expressed as the average number of analgesic segments on each side. The volume of 2% mepivacaine per spinal segment (dermatome) (ml D^{-1}) (the extradural dose requirement) was calculated using the formula: amount of 2% mepivacaine injected (15 ml) divided by number of spinal segments blocked. After assessment of the spread of analgesia, the patient was anaesthetized. Upper abdominal surgery was performed under combined extradural and general anaesthesia.

The relation between age and extradural dose requirement was evaluated by linear regression analysis. The threshold for statistical significance was set at $P < 0.05$.

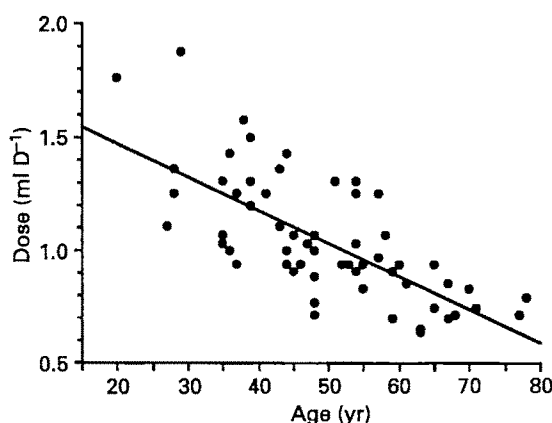


FIG. 1. Relationship between age and extradural dose requirement in thoracic extradural anaesthesia. D = Dermatome. Regression equation (straight line): $y = -0.015x + 1.266$; $r = -0.70$, $P < 0.001$.

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Mean (SD) age, weight and height were 49.2 (12.7) yr, 56.1 (8.7) kg and 1.61 (0.08) m, respectively. Fifteen millilitre of 2% mepivacaine blocked 15.5 (4.2) spinal segments. The calculated extradural dose requirement decreased with ageing ($r = -0.7$, $P < 0.001$) (fig. 1), average extradural dose requirements being: age 20–29 yr ($n = 5$)—1.47 (0.33) ml D⁻¹; 30–39 yr ($n = 11$)—1.24 (0.21) ml D⁻¹; 40–49 yr ($n = 19$)—1.02 (0.21) ml D⁻¹; 50–59 yr ($n = 14$)—1.03 (0.19) ml D⁻¹; 60–69 yr ($n = 9$)—0.78 (0.12) ml D⁻¹; 70–79 yr ($n = 4$)—0.77 (0.05) ml D⁻¹. The extradural dose requirement in the elderly (60–79 yr) was smaller than that in young adults (20–39 yr) by about 40%.

COMMENT

We have shown that a fixed dose of local anaesthetic administered into the thoracic extradural space blocked more segments in older patients compared with younger subjects.

Schulte-Steinberg, Ostermayer and Rahlfs [1] studied the spread of midthoracic extradural anaesthesia with 1% etidocaine and reported little correlation between age and spread of analgesia. The volume given in their study varied between 7 and 10 ml according to age and height: the older and smaller patients received the smaller doses of local anaesthetic. Their results are not directly comparable with ours, because the volume injected in our study was fixed. Furthermore, the method of administration, through a catheter or extradural needle, may affect the spread of local anaesthetic in the extradural space.

Leakage of anaesthetic solution from the extradural space may vary considerably in different spinal segments. Recent studies indicate that an increasing dose of anaesthetic solution resulted in greater leakage and consequently in greater calculated extradural dose requirement [5, 6]. The dependency of extradural dose requirement on volume injected makes evaluation of dose requirement difficult. We consider that extradural dose requirement calculated for a fixed dose may be adequate to evaluate the intrinsic factors that affect extradural spread of anaesthetic.

This was an unblinded study using a fixed dose of local anaesthetic. If two different volumes had been compared and the observer blinded to the volume used, a greater correlation between age and extradural dose requirement might have been obtained. Blinded studies should be performed in future.

Measurements were taken 15 min after extradural administration in this study. Although the maximum spread of extradural analgesia with 2% mepivacaine occurred at 20–30 min, the greater part of this maximum spread was already blocked at 15 min. The T9–10 interspace was identified by examination and palpation of the spine. It is possible that there was some error in identifying the exact interspace. However, this should not affect our conclusions, as we assessed both upper and lower borders of analgesia instead of the height of analgesia.

Despite the use of a fixed dose and constant rate administration by syringe pump, there was a large variation in the spread of analgesia and several factors other than age may affect the spread of local anaesthetic within the extradural space. However, an age-related decrease in thoracic extradural dose requirement was demonstrated. A reduction of the dose in thoracic extradural anaesthesia in the elderly is recommended.

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ACTIONS OF THE HYPNOTIC ANAESTHETIC, DEXMEDETOMIDINE, ON NORADRENALINE RELEASE AND CELL FIRING IN RAT LOCUS COERULEUS SLICES

C. M. JORM AND J. A. STAMFORD

SUMMARY

We have examined the effects of the highly selective α_2 -adrenoceptor agonist, dexmedetomidine, on noradrenaline release and cell firing in isolated, superfused slices of rat locus coeruleus. Dexmedetomidine decreased both noradrenaline release and cell firing rate in a concentration-dependent fashion, with an EC_{50} of 3.97 (SEM 0.97) $\times 10^{-9}$ mol litre $^{-1}$ for noradrenaline release and 0.92 (0.53) $\times 10^{-9}$ mol litre $^{-1}$ for unit activity. Both effects were reversed completely by the selective α_2 antagonist, atipamezole 10^{-6} mol litre $^{-1}$. These results suggest that cell firing and noradrenaline release are under α_2 receptor control and that dexmedetomidine potently stimulates these receptors. We conclude that these effects are consistent with the locus coeruleus being a major site of action of the hypnotic anaesthetic α_2 agonists. (Br. J. Anaesth. 1993; 71: 447-449)

KEY WORDS

Sympathetic nervous system, pharmacology: dexmedetomidine, atipamezole.

Over the past 2 years, there has been increasing interest in the potential of α_2 adrenoceptor agonists, particularly the highly selective drug, dexmedetomidine. It has been suggested that the locus coeruleus may be an anatomical site of action for these agents [1]. It is the major noradrenergic nucleus, has diffuse ascending and descending projections, and controls (*inter alia*) vigilance.

Our laboratory has recently [2] developed a method for simultaneously monitoring single cell activity and neurotransmitter release in brain slices using a single carbon fibre microelectrode (CFM). This combination makes it possible to investigate quantitatively the relationship between the noradrenaline release and cell excitation or inhibition.

Most previous experimental work on α_2 agonists has centred on the pharmacological modulation of post-anaesthetic sleep times. Our aim was to examine the effects of dexmedetomidine on individual locus coeruleus neurones and to attempt to relate, for the first time, actions on neuronal activity and release of noradrenaline.

METHODS AND RESULTS

Rat brain slices 350 μ m thick were prepared and maintained as described previously [3]. The locus coeruleus was visualized as a translucent oval area on the ventrolateral border of the fourth ventricle. A CFM was then lowered into the slice until a spontaneously active single unit could be discriminated. Cell identity was further confirmed by the standard biphasic potentials and a firing rate of 0.2-5.0 Hz.

Quantitative, real-time release of noradrenaline was measured using fast cyclic voltammetry (FCV) as described previously [4]. An input voltage of 1.5 cycles of a triangular waveform (-1.0 to +1.4 V vs Ag/AgCl) at a scan rate of 480 V s $^{-1}$ was applied to the potentiostat at a rate of 2 s $^{-1}$. A sample-and-hold circuit monitored the current at the oxidation potential for noradrenaline (+600 mV).

Between voltammetric scans, the CFM monitored the voltage at the microelectrode tip. This voltage was amplified and filtered, resulting in an electrophysiological signal with 50-ms gaps occurring at each voltammetric scan. Cells were counted with an integrating ratemeter. A dual-channel chart recorder was used to record continuously both firing rate and stimulated release of noradrenaline.

Trains of 20 pulses (10 mA, 0.2 ms, 200 Hz) were applied every 20 min. The rate of cell firing was measured for the 2 min immediately before stimulation. A pre-drug period of 1 h was used for each experiment; three measurements of noradrenaline release were made in this period. Drug effects on release of noradrenaline and cell firing were subsequently calculated relative to this initial period. This required that the cell would fire regularly for 60 min. Cells that did not maintain this were discarded. All experiments commenced within 3 h of the dissection. Control studies ($n = 8$) were performed in which no drug was added and firing rate and release monitored over a 200-min period.

Incremental concentrations of dexmedetomidine 10^{-11} , 10^{-10} , 10^{-9} , 10^{-8} and 10^{-7} mol litre $^{-1}$ were added at 20-min intervals, immediately after the

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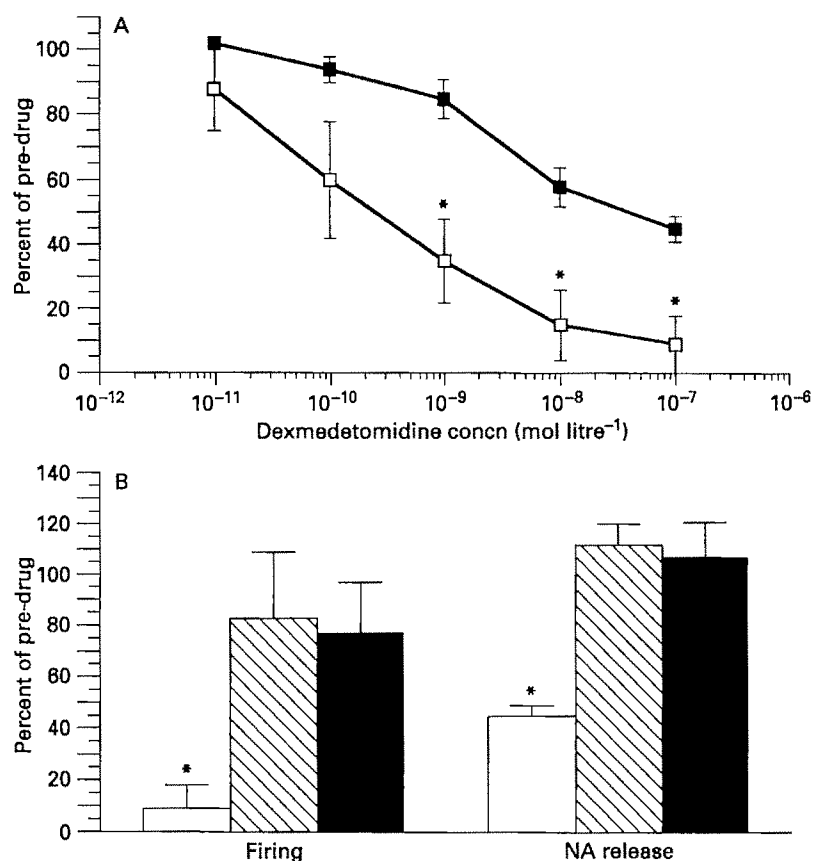


FIG. 1. A: Concentration-response curves for the reduction in cell firing rate (□) and release of noradrenaline (■) in the locus coeruleus caused by dexmedetomidine (mean, SEM; $n = 7$ slices). * $P < 0.05$: cell firing *vs* noradrenaline release (paired t test). B: Reversal of effect of dexmedetomidine 10^{-7} mol litre $^{-1}$ by atipamezole 10^{-6} mol litre $^{-1}$ (mean, SEM). □ = Dexmedetomidine ($n = 7$); ▨ = dexmedetomidine + atipamezole ($n = 7$) (■ = controls ($n = 8$)). * $P < 0.05$: dexmedetomidine *vs* all other groups (ANOVA). No other significant differences between groups.

stimulation that represented the effect of the previous concentration on noradrenaline release. After the effect of dexmedetomidine 10^{-7} mol litre $^{-1}$ was recorded, atipamezole 10^{-6} mol litre $^{-1}$ was added (to the dexmedetomidine) for a further 40 min to assess reversal. The electrodes were calibrated in noradrenaline 5×10^{-7} mol litre $^{-1}$ at the end of the experiment.

The effects of dexmedetomidine on release of noradrenaline and cell firing were compared by paired t test. Comparison of control, dexmedetomidine and dexmedetomidine with atipamezole groups was by analysis of variance (ANOVA).

In the 15 slices examined, the mean cell firing rate was 1.7 Hz (range 0.64–4.30 Hz). Mean stimulated release of noradrenaline corresponded with a peak extracellular concentration of noradrenaline 2.1 (SEM 0.4) $\times 10^{-7}$ mol litre $^{-1}$. The response of the cells to the stimulations was variable, although the most common pattern was a brief excitation, followed by inhibition and then return to the baseline rate of firing. Release of noradrenaline and cell firing in control slices were generally stable over this quite long period of study (firing rate at 160 min being 86 (9)% of that in the initial 1 h, and noradrenaline release 110 (6)%).

Dexmedetomidine reduced significantly both cell firing and release of noradrenaline in a concentration-dependent manner (fig. 1A). The maximum

decrease in release of noradrenaline was 55 (4)% whilst, for six of seven cells, dexmedetomidine completely abolished cell firing. EC_{50} for dexmedetomidine was 3.97 (0.97) $\times 10^{-9}$ mol litre $^{-1}$ on release of noradrenaline and 0.92 (0.53) $\times 10^{-9}$ mol litre $^{-1}$ on unit activity. Greater concentrations of dexmedetomidine induced a significantly ($P < 0.05$: paired t test) greater inhibition of cell firing than of release of noradrenaline.

The actions of dexmedetomidine 10^{-7} mol litre $^{-1}$ on noradrenaline release and unit activity were reversed completely by simultaneous administration of atipamezole 10^{-6} mol litre $^{-1}$ for 40 min, with no significant difference (ANOVA) from time-matched controls (fig. 1B).

COMMENT

Locus coeruleus neurones fire regular spontaneous action potentials in brain slice preparations for many hours [5]. The firing reflects a balance between a persistent inward sodium current active near the action potential threshold and a calcium-dependent potassium current activated after the action potential (causing an after-hyperpolarization). Known inhibitors include GABA, glycine and noradrenaline, while glutamate is excitatory. *In vivo*, the pattern of firing is more complex, as it is affected markedly by external stimuli, sleep etc.

The results of this study show that dexmedetomidine reduces both release of noradrenaline and the basal firing rate of locus coeruleus cells. The complete reversal of the effect by atipamezole, a highly selective α_2 antagonist, is strong evidence that both effects are mediated via α_2 receptors. The maximum response to α_2 receptor stimulation differs for the two indices: cell firing appears more sensitive, but also a more variable index of α_2 activity than release of noradrenaline.

It appears, on the basis of catecholamine depletion studies [6], that, in addition to the effects of the α_2 agonists on *presynaptic* α_2 receptors, some of the anaesthetic effects of dexmedetomidine result from action on *postsynaptic* α_2 receptors. The present study nevertheless shows that dexmedetomidine has potent actions in the locus coeruleus, where it acts presumably on dendritic (i.e. *pre-synaptic*) α_2 receptors, and supports the view that the locus coeruleus may be a major site of action of this drug. The apparent dissociation of the actions of the drug on cell firing and noradrenaline release may indicate different receptor subtypes or receptor reserves for these processes. This is currently under investigation.

ACKNOWLEDGEMENTS

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DISCREPANCY BETWEEN THE DEVELOPMENT OF TOLERANCE TO BUPIVACAINE IN EXTRADURAL AND SPINAL ANAESTHESIA IN RABBITS

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SUMMARY

We gave equal groups of rabbits seven extradural ($500 \mu\text{g kg}^{-1}$) or intrathecal ($250 \mu\text{g kg}^{-1}$) injections of bupivacaine, at 24-h intervals. A decrease in the duration of motor block was observed after the extradural injections. The intrathecal injections exerted a reproducible effect. An additional regimen was tested in which five doses of bupivacaine $125 \mu\text{g kg}^{-1}$ were administered intrathecally after a loading dose of $250 \mu\text{g kg}^{-1}$, when the animals showed partial recovery from the previous dose; there was no decrease in the effect. The absence of tolerance to intrathecal bupivacaine implies that tachyphylaxis to extradural local anaesthetics results from a decrease in availability of the drug to the neural target, rather than a diminution in effect at the site of action. (Br. J. Anaesth. 1993; 71: 450–452)

KEY WORDS

Anaesthetic techniques: spinal, extradural. Anaesthetics, local: bupivacaine. Tachyphylaxis.

Tachyphylaxis to local anaesthetics has been described widely when different routes of administration are used, including extradural injections [1]. However, in several studies the development of tolerance has not been confirmed after continuous or repeated spinal administration of local anaesthetics [2, 3]. As the indications for extradural and spinal analgesia in many cases are similar, we designed this study to examine and compare the existence and extent of tolerance to a local anaesthetic in the extradural and spinal spaces. Local anaesthetic action, evaluated by estimation of motor block duration, has been assessed after repeated intrathecal and extradural injections of bupivacaine in rabbits.

METHODS AND RESULTS

Seven injections of bupivacaine were administered to two equal groups of male rabbits (2.8–3 kg; $n = 6$) extradurally (group I; bupivacaine $500 \mu\text{g kg}^{-1}$) or intrathecally (group II; bupivacaine $250 \mu\text{g kg}^{-1}$) at 24-h intervals via chronically implanted catheters. Cannulations were performed using modifications of techniques described elsewhere [4, 5]. The period between administration of the drug and restoration of the animal's ability to move freely was defined as the duration of motor block.

We found a significant gradual decrease in the duration of the motor block produced by repeated extradural injections (group I) commencing from the fourth injection ($P < 0.05$ between the 3rd and 4th days; $P < 0.01$ between 4th–5th, 5th–6th and 6th–7th days) (fig. 1). In contrast, animals treated by repeated intrathecal injections of the anaesthetic (group II) exhibited similar periods of motor block throughout the 7 days of the study (fig. 1).

As tolerance to repeated intrathecal injections of bupivacaine at 24-h intervals was not found, an additional study was undertaken, in which bupivacaine was injected before the animal exhibited complete recovery from the effect of the previous dose. Six rabbits (group III) received bupivacaine $250 \mu\text{g kg}^{-1}$ followed by five doses of $125 \mu\text{g kg}^{-1}$. These doses were administered as soon as the animal supported the back of the body using four limbs (showed partial recovery from the block). The period between injection and restored ability of the animal to stand, still exhibiting ataxia, was referred to as the duration of maximum motor block. The repeated intrathecal injections of bupivacaine at short intervals (each injection was administered before complete recovery from the effect of the previous dose) exerted a similar maximum motor block (fig. 1).

COMMENTS

Tachyphylaxis to extradurally administered local anaesthetics has been described in humans [1]. Tachyphylaxis to spinal administration of local anaesthetics has also been assumed, based on two types of evidence [1]. The first was the finding of a reduction in the duration of analgesia produced by successive subarachnoid injections of lignocaine in patients with crural gangrene caused by vascular occlusion. This reduction was attributed to tolerance. However, an alternative explanation, relating the reduction in effect to an increase in pain because of progression of the pathological process, may be

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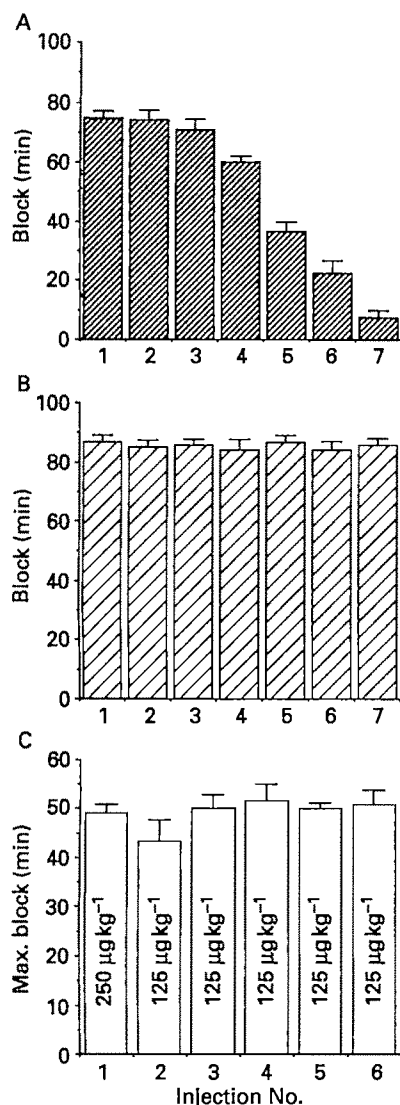


FIG. 1. Duration of motor block produced by repeated injections of bupivacaine. The serial number of the injection is presented on the horizontal axis, and the mean duration of motor block on the vertical axis. Error bars represent SEM; $n = 6$. A: Repeated extradural injections of bupivacaine $500 \mu\text{g kg}^{-1}$ at 24-h intervals. The duration of motor block refers to the period between the injection and the restored ability of the animal to move freely without limitations. $P < 0.05$ between the 3rd and 4th days; $P < 0.01$ between 4th–5th, 5th–6th and 6th–7th days. B: Repeated intrathecal administration of bupivacaine $250 \mu\text{g kg}^{-1}$ at 24-h intervals. Duration of motor block defined as in the extradural group (A). C: Repeated intrathecal injections of bupivacaine $125 \mu\text{g kg}^{-1}$ after a loading dose of $250 \mu\text{g kg}^{-1}$. Each injection was performed when animals had recovered partially from the block. The duration of maximum motor block refers to the period between the injection and the restored ability of the animal to stand on four limbs, still exhibiting ataxia.

proposed. The second type of evidence was based on the observation that the pH of the CSF decreases after repeated spinal administration of local anaesthetics. It was hypothesized that this pH reduction affected distribution of the agent between CSF and nerve tissue, causing tolerance. However, the finding was not accompanied by an estimation of the pharmacological effect. A reduction in the duration of the effect of these anaesthetics after caudal administration was presented as evidence of tachyphylaxis to intrathecal administration. As different anatomical structures affect drug distribution in

spinal compared with caudal anaesthesia, the legitimacy of this evidence is doubtful.

Analysis of results obtained by Kroin and colleagues, who used continuous subarachnoid administration of bupivacaine in dogs for 16 weeks, did not show a decrease in motor block [2]. Successful analgesia of 7 months duration, achieved by bupivacaine in a patient with a spinal tumour, has been reported [3]. In the present study, repeated spinal injections of bupivacaine at 24-h intervals produced reproducible effects. Repeated injections of the drug before full recovery from the previous dose also did not show any reduction in effect.

A decrease in effect after repeated extradural administration of local anaesthetics may occur because of a fibrosis around the catheter. Formation of connective tissue in the extradural space was reported in rats [5]. Moreover, a study in cancer patients after prolonged spinal administration of bupivacaine and morphine revealed formation of adhesions mostly in the extradural space [6]. Obviously, this mechanism cannot account for the reduction in anaesthetic efficacy when short intervals between injections were used. A decrease in the duration of block has been reported when local anaesthetics were administered extradurally shortly after the reduction of the effect of the previous injection [1]. However, in the same work it was shown that after three or four injections the reduction in anaesthetic efficacy did not continue. Moreover, if repeated injections were given before the end of the effect of the previous dose, it resulted in augmentation of effect [1]. We speculate that frequent extradural injections of anaesthetic solutions may increase the amount of extracellular and intracellular fluid in the area of injection or decrease the buffering capacity and pH of the surrounding tissue. This may reduce the availability of the drug to nerve cells, causing a reduction in effect. Hence, repeated extradural administration of local anaesthetics may display different patterns of effect:

- (1) Initial diminution as a result of decreased drug availability to nerve cells because of changes in pH and accumulation of fluids around the area of injection.
- (2) Initial augmentation because of anaesthetic accumulation when administered before elimination of the residual drug from the nerve cells.
- (3) Delayed diminution after a few days because of formation of adhesions around the catheter.

The basis for tolerance could be a reduction either in the effect at the receptor level or in the fraction of anaesthetic reaching the neural target. The molecular mechanism of anaesthetic action on neural tissue is similar for both spinal and extradural anaesthesia. The difference in the development of tolerance between the routes of administration implies that tachyphylaxis to local anaesthetics results from a decrease in drug availability, rather than alteration in the effect at the site of action.

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TABLE I. Part of questionnaire distributed to each anaesthetist employed at the departments

1. What magnitude of aberrance from the vaporizer dial setting do you find acceptable?	0% rel.	0-5% rel.	5-10% rel.	10-20% rel.	> 20% rel.
	☐	☐	☐	☐	☐
	☐ Dial setting irrelevant, dosage according to clinical parameters.				
2. Are you using Isotec or Fluotec vaporizers (Tec 3) with the dial set between the fixed marks in the dial setting range 0-1%?	☐ Often	☐ Seldom/never			
3. Are you using Enflurtec vaporizers with (Tec 3) the dial set at 0.2/0.4/0.6/0.8%?	☐ Often	☐ Seldom/never			
4. If you have answered seldom/never to question 2, is the reason that you	☐ do not trust the vaporizer at these dial settings ☐ do not find these dial settings clinically relevant ☐ do not use this type of vaporizer				
5. If you have answered seldom/never to question 3, is the reason that you	☐ do not trust the vaporizer at these dial settings ☐ do not find these dial settings clinically relevant ☐ do not use this type of vaporizer				

"half" and "full" marks. All were transported upright and with the dial in the "off" position. Each vaporizer was mounted on an anaesthetic machine. If this was done using the Selectatec system, we ensured that the manifold port valve "O" rings were intact, and the lever-lock was in the "lock" position. Similarly, for every vaporizer we checked that the drain port was fully closed.

At the vaporizer outlet, an ISO-cone with a 50-cm p.v.c. hose was mounted. The sampling tube of the monitor was connected via a Y-piece at the end of the hose, thus providing a homogenous gas mixture and a free outlet, avoiding pressure variations. Before each test, the vaporizer was flushed with the dial set at 4% and an oxygen flow of 8 litre min⁻¹ for a few minutes, in order to remove any nitrous oxide dissolved in the anaesthetic liquid, as this may have a major influence on output [19]. In addition, we monitored the eventual output of nitrous oxide during the test sequence. The vaporizers were tested at three different flow rates of oxygen: 0.5, 2 and 6 litre min⁻¹. Fluotec and Isotec vaporizers (Tec 3, Ohmeda Ltd, U.K.) had the dials set at 0.5, 1, 2, 3.5 and 5%. As these vaporizers are used commonly with the dial set between the fixed marks, they were tested also at settings of "0.25%" and "0.75%". Transparent plastic scales (log scale) with marks representing 0, "0.25" and "0.75" (log centre) settings for each type of vaporizer were produced. The 0 marks of the plastic scale and the dial were aligned and the dial was then set at "0.25" or "0.75", thus testing each vaporizer under uniform conditions. Enflurtec vaporizers (Tec 3, Ohmeda Ltd, U.K.) had the dials set at 0.2, 0.4, 0.6, 0.8, 1, 2, 3.5 and 5%.

The output was noted at each dial setting and flow rate, and readings were made continuously until stability was reached, usually after 30 s. The date of the last service check was also noted. After the test sequence at each department, the Brüel & Kjær Anaesthetic Gas Monitor Type 1304 was checked against the calibration gas in order to evaluate any drift. Before our investigation of the vaporizers, we distributed a questionnaire to each anaesthetist employed at the departments (table I).

Maintenance policy

According to guidelines issued by the Danish National Board of Health, all vaporizers should be

sent annually to the manufacturer for a complete service check; this includes disassembling, cleaning and calibration. All departments stated that these guidelines had been followed. Apart from this, one department had implemented 3-monthly calibration checks using an anaesthetic agent monitor (Datex Capnomac, Datex, Finland). If the vaporizer output exceeded some specific limits ($\pm 20\%$ rel. in the dial setting range 0-1%, $\pm 15\%$ rel. in the dial setting range 2-4%), the vaporizer was sent for recalibration. Oxygen was used as carrier gas at a flow rate of 5 litre min⁻¹.

Criteria for acceptance

Because of lack of explicit data (in the Operators' Manuals) regarding the actual calibration limits, we decided that the output was acceptable if it was within $\pm 10\%$ rel. of the dial setting. Added to this was the error in measurement, caused by the gas monitor and calibration gas ($\pm 5\%$ rel.). As a consequence, our acceptance limits were $\pm 15\%$ rel. (or ± 0.1 vol%, greatest value chosen). As the Fluotec 3 and Isotec 3 vaporizers do not have fixed marks at dial settings "0.25" and "0.75", the outputs at these dial settings are not included in the part of the investigation dealing with acceptability. Consequently, the Fluotec and Isotec vaporizers were tested for acceptability at five dial settings and the Enflurtecs at eight dial settings; all vaporizers at three flow rates.

Statistical analysis

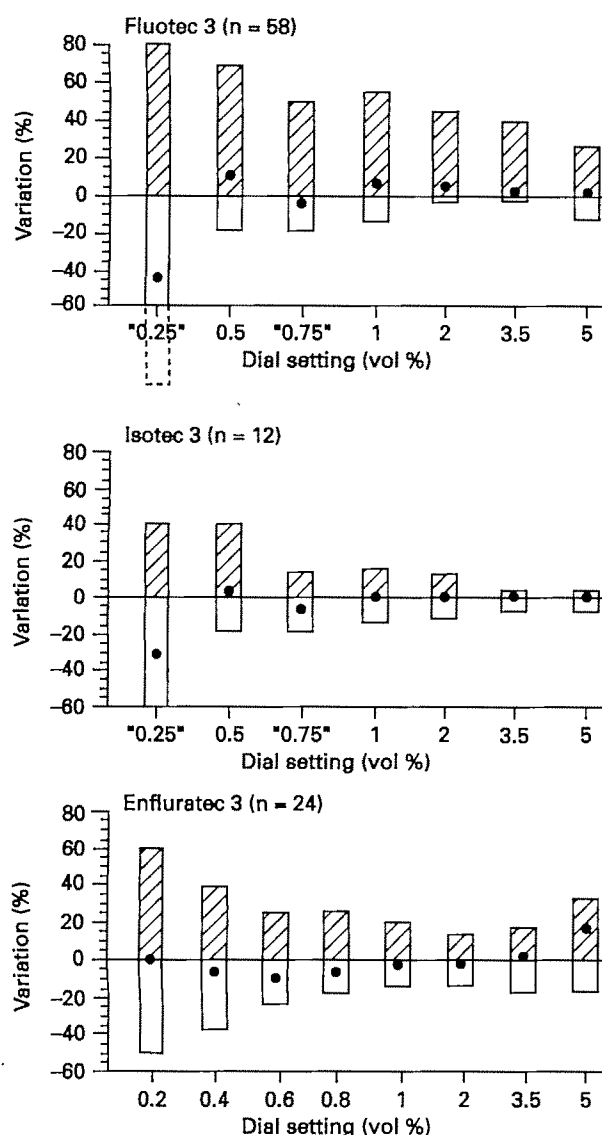
The accuracy of the Tec vaporizers undergoing quarterly calibration checks and of those submitted only to annual service and calibration was compared using chi-square analysis, as was the discrepancy between answers in the questionnaire. $P < 0.05$ was regarded as statistically significant.

RESULTS

A total of 114 vaporizers was reported to be in clinical use at the seven departments. One hundred and two vaporizers (90%) were tested; the rest were in use for anaesthetic procedures at the time of our visit. We decided to omit the data for two Penlon vaporizers and seven Dräger Vapor 19.n vaporizers because of small numbers. Data for the equipment tested are given in table II. The date for next

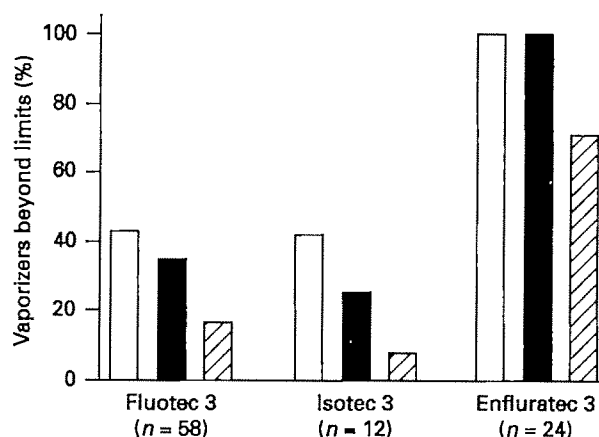
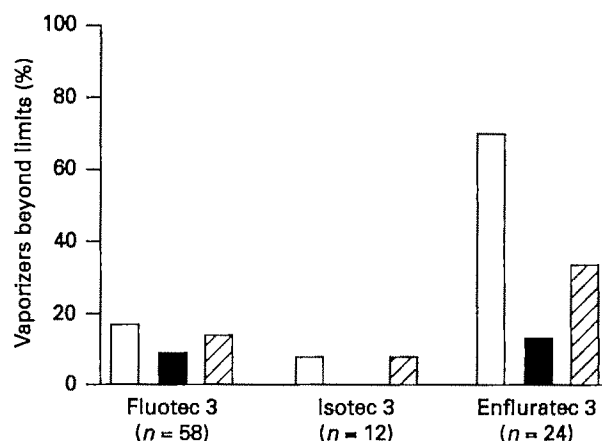
TABLE II. Type of vaporizers and time from last calibration (number or mean (range))

Vaporizer	Number studied	Time from last calibration (months)
Fluotec 3 (5%)	58	7.0 (2-16)
Isotec 3 (5%)	12	9.0 (1-12)
Enflurtec 3 (5%)	24	8.6 (1-12)


 FIG. 1. Relative variation from dial setting in Tec 3 vaporizers. Each column shows range of relative variation (● = mean) at fixed vaporizer settings. Carrier gas: oxygen 6 litre min⁻¹.

calibration was overdue in three vaporizers (by 1, 2 and 4 months). The mean age of the Tec vaporizers (time in use) was approximately 10 yr.

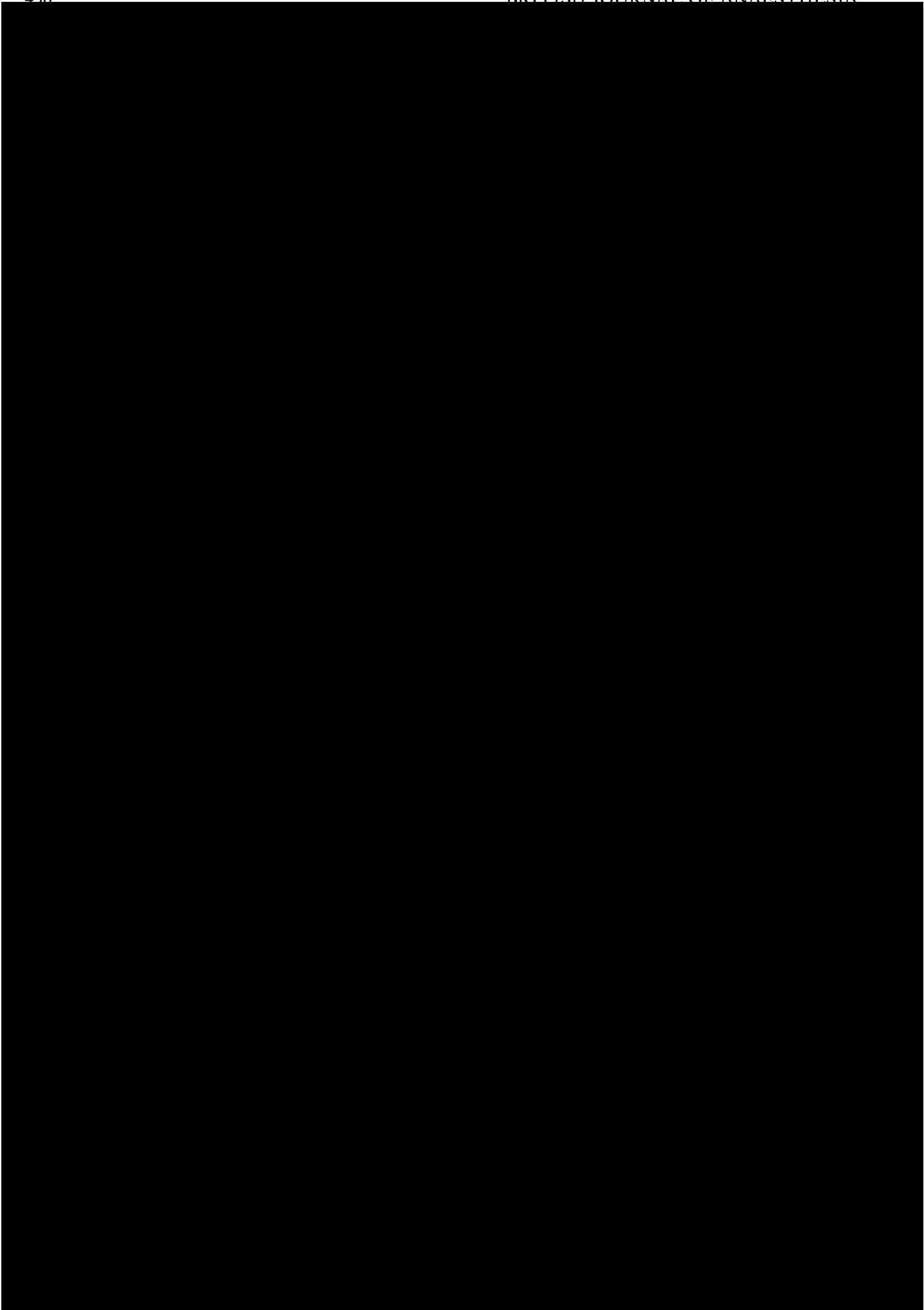
The department using 3-monthly calibration checks—performed by their own medical physics staff—stated that two vaporizers (of a total of 33 Tec 3) had been sent for recalibration during the past year. Figure 1 illustrates the relative range and mean deviations of the vaporizer output from the dial settings at a flow rate of 6 litre min⁻¹. The range of outputs, for one type of vaporizer and one dial setting, was largest with the Fluotec 3 (e.g. dial set to 1%: output 0.85–1.55%) and smallest with the


 FIG. 2. Vaporizers giving outputs beyond the acceptance limits ($\pm 15\%$ rel.) at one or more combinations of the five or eight dial settings (0.2–5%) and the three flow rates of oxygen: 0.5 litre min⁻¹ (□); 2 litre min⁻¹ (■); 6 litre min⁻¹ (▨).

 FIG. 3. Vaporizers giving outputs beyond the acceptance limits ($\pm 15\%$ rel.) under various conditions, but all at 6 litre min⁻¹ flow. □ = Beyond limits at any one or more of the five or eight dial settings; ■ = beyond limits at any two or more of the five or eight dial settings; ▨ = beyond the limits at the dial settings 0.5%, 1% and 2% (Fluotec 3 and Isotec 3) or 0.6%, 1% and 2% (Enflurtec 3).

Isotec 3 (dial set to 1%: output 0.85–1.15%). Using the Fluotec 3 and Isotec 3 vaporizers with the dial set at “0.25”%, the mean output was substantially less than the dial setting, and the relative range of outputs was wider, as in the fixed settings. Only one of the Fluotec 3 vaporizers gave no output at the dial setting “0.25”%; thus a “cut off” phenomenon was not demonstrated generally. When the dial was set at “0.75”%, the vaporizer performance was of the same relative proportion as in the fixed settings.

A small flow dependency was seen with the Tec 3 vaporizers, giving excessive output at the greatest dial setting and slowest flow rate; this diminished as the flow increased. The output was not influenced by changes in flow rate (0.5–6.0 litre min⁻¹) at dial settings most relevant clinically (0.5–2%).

Figure 2 illustrates the number of vaporizers with an unacceptable inaccuracy at any one of the 15 or 24 combinations of flow and vaporizer setting (Fluotec and Isotec, three flow rates and five dial settings; Enflurtec, three flow rates and eight dial settings). In order to derive the percentage of vaporizers with an unacceptable output, especially at clinically



substantial variation: 10% of the Fluotec 3 were in this group, compared with none of the Isotec 3 and 15% of the Enflurtec 3. The second condition was output beyond the acceptance limits in the clinically relevant dial setting range (0.5 or 0.6%, 1%, 2%; three data points for each vaporizer); on this basis, 15% of the Fluotec 3, 10% of the Isotec 3 and 35% of the Enflurtec 3 delivered excessive output.

One reason for the greater accuracy of the Isotec 3 vaporizers could be that, because of the cost of isoflurane, these have been used less frequently. Only a relatively small number (12) of Isotec 3 vaporizers was investigated. As the precise measurement of anaesthetic agent concentration in the gas mixture delivered to the patient during anaesthesia is still not routine, the accuracy of anaesthetic agent vaporizers is important. While cardiovascular depression caused by excessive vaporizer output may be recognized and corrected, it is better avoided altogether.

Carter and colleagues [17] examined 631 maintenance records of vaporizers in the West of Scotland; among these were 236 Fluotec 3 and 214 Enflurtec 3. All vaporizers were given 6-monthly calibration checks; apart from this there was no uniform policy for maintenance. Oxygen was used as carrier gas at 5–7 litre min⁻¹ and the monitors were Riken interferometers. The acceptance limits were $\pm 10\%$ rel., and to this was added the error of measurement. They demonstrated calibration errors in 9% of the Fluotec 3 and in 4% of the Enflurtec 3. Isotec 3 vaporizers were not examined. More vaporizers failed the acceptance limits in our study, although the acceptance limits were identical and the flow rates similar. The apparently smaller variation in the output found by Carter's group could be a result of the procedure of 6-monthly calibration checks and recalibration of the vaporizers failing the acceptance limits, compared with the general policy of maintenance revealed in our study, namely annual service and recalibration. In addition, the vaporizers we investigated had been in use for 10 years, as opposed to 4 years in use for the vaporizers investigated by Carter and colleagues.

At the one department in our study which had implemented 3-monthly calibration checks, the accuracy of the vaporizers was significantly better. Because of the smaller resolution of the monitor used for measurement at this department, compared with that used in the present study, the limits were somewhat less restricted in the dial setting range 0–1%, and measurements at 5% were not performed.

The mean output of the Fluotec 3 and the Isotec 3 vaporizers with the dial setting between the fixed marks of 0%, 0.5% and 1%, was somewhat less than that expected, especially at "0.25%". This could be attributable to the fact that the vaporizers were not calibrated at these points. We could not demonstrate the "cut-off" phenomenon found by Latta [5] using Fluotec 3 vaporizers with the dial setting positioned midway between "OFF" and 0.5%, although there might be a difference in the exact position of the dial setting. As the survey among the

anaesthetists at the seven departments showed that the Fluotec 3 and Isotec 3 vaporizers were used quite often (39%) with the dial setting positioned between the fixed marks in the low concentration range, we recommend calibration and marking of the "0.25%" and "0.75%" set points.

From our results we believe it is reasonable to presume that all vaporizers could achieve acceptable accuracy by implementing regular field calibration checks, and sending for recalibration only those vaporizers with output beyond certain limits. However, the regular service check of the vaporizers with acceptable output should not be omitted, but the interval extended.

ACKNOWLEDGEMENTS

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CORRESPONDENCE

PRE-EMPTIVE ANALGESIA—USE OF IMMEDIATE EARLY GENES EXPRESSION AS MARKERS OF NEURONAL STIMULATION

Sir,—We were interested in the article on pre-emptive analgesia by Dahl and Kehlet [1] and comments made in a letter on this same topic by Hardy [2]. Dahl and Kehlet concluded that most trials of pre-emptive analgesia in man do not show a completely convincing clinical effect and suggest "further investigation before clinical recommendations of pre-emptive analgesia can be established". While we share some of Dr Hardy's misgivings that much of the scientific justification quoted for the pre-emptive approach to analgesia comes from work on the electrophysiological phenomenon of "wind-up" which occurs in the spinal cord only in the *short term* after nociceptive stimulation of sensory fibres, we would disagree strongly with his conclusion that "It is only by the study of pain and pain mechanisms in man that there will be any advances in clinical practice" [2]. We believe that experimental studies of "immediate early gene" expression in spinal cord after noxious stimulation can already answer some of the questions regarding the ineffectiveness of many pre-emptive analgesia regimens with drugs used currently.

The primary afferent sensory barrage that strikes the spinal cord at the time of injury not only produces immediate electrophysiological wind-up, but also initiates a set of *long-term* molecular events, including the expression of immediate early genes (IEG). Even a transient noxious stimulation leads to the expression of the IEG *c-fos* within 10 min of stimulation in the superficial layers of the dorsal horn of the spinal cord [3]. These layers are known to receive nociceptive afferent input and 50% of the neurones which expressed Fos protein had connections to known higher pain centres [4]. There is evidence that IEG expression has functional and behavioural correlates. Fos protein dimerizes with another IEG protein termed Jun to bind to a particular site on DNA known as AP1, leading to altered gene transcription [5]. The genes for nerve growth factor and pre-prodynorphin have the AP1 binding sites, and Fos protein has been shown to co-localize with dynorphin protein [6]. Models of both acute pain and inflammation have been associated with increased dynorphin production and dynorphin causes hyperalgesia when applied directly to the spinal cord [7]. The "pain" behaviour in response to noxious stimulation in a rat model has been shown to be directly proportional to the quantity of Fos expression [8].

Study of these IEG may explain why pre-emptive analgesia may be difficult to obtain. It has been shown that morphine can suppress electrophysiological wind-up with 10-fold less drug when given pre-emptively rather than after the stimulation [9]. In both situations, however, there is still a significant level of induction of *c-fos* with noxious stimulation [10]. Furthermore, MK-801, a competitive NMDA antagonist, also has been shown to block wind-up, but again it does not fully block *c-fos* expression [11]. Thus in two situations in which wind-up measurements might indicate that pre-stimulus dosing with morphine or the use of NMDA antagonists might be effective as pre-emptive analgesics, IEG studies, in contrast, have indicated that the long-term molecular changes still occur. These latter changes may be associated with hyperaesthesia and allodynia [7]. The study of IEG may also explain why a pre-emptive approach to analgesia may be ineffective in surgery for previously painful pathology. Noxious stimulation *anywhere* in the body has a "priming" effect on the spinal cord, allowing further noxious stimulation, for example surgery, to cause dramatically increased induction of *c-fos* in the spinal cord [12]. Present regimens of analgesia often do not fully block noxious stimulation at the time of surgery or, alternatively, the analgesia wears off too quickly, allowing a barrage of nervous impulses again to induce *c-fos*. Alternative approaches are required. There is evidence that high-dose pre-emptive i.v. magnesium may have a significant analgesic effect [13] by its block of the NMDA channel. A similar strategy with non-competitive NMDA antagonists such as CPP (3-[2-carboxypiperazin-4-yl]propyl-1-phosphonic acid) [14], may also prove useful. The multi-modality approach to pre-emptive analgesia as suggested previously by Kehlet himself, using these alternative

pharmacological strategies, may allow us to achieve the cherished goal of pre-emptive analgesia. Immediate early gene research complements the established neurophysiological studies and the use of these markers may lead not only to a greater understanding of the pathogenesis and treatment of acute pain, but also to an understanding of the transition between acute and chronic pain [15].

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INFLUENCE OF CARDIAC OUTPUT ON THE CORRELATION BETWEEN MIXED VENOUS AND CENTRAL VENOUS OXYGEN SATURATION

Sir,—Because there was close correlation between mixed venous ($\bar{Sv}_{O_2}$) and central venous (Scv_{O_2}) oxygen saturations in his study of largely elective cardiac surgical patients, Dr Berridge [1] asserted that Scv_{O_2} may be used to estimate derived oxygen transport variables in emergency situations. This is in disagreement with previous studies [2] and, we feel, merits further comment.

Statistically, close correlation between two measurements implies that there is a mathematical relationship between them, not that they are interchangeable. For example, if there had been a constant difference of, say, 20% between $\bar{Sv}_{O_2}$ and Scv_{O_2} in all patients, the correlation coefficient would have been 1.0. Clearly, however, the two measurements could not be said to be interchangeable. A more useful test to determine whether two measurements are interchangeable is the method described by Bland and Altman [3]. This examines the differences between measurements made in an individual patient and may be applied to the data in Dr Berridge's paper. The 95% confidence intervals for the mean (mean $\pm$ 2 SEM) of the difference between central and mixed venous oxygen saturation for the entire study group were ($Scv_{O_2} - \bar{Sv}_{O_2}$) = 2.45–3.75%. To calculate the potential variation in the difference between central and mixed venous oxygen saturation for an individual patient in the study group, we need to calculate SD. The SD of the entire study group is calculated from the formula: $SEM = SD / \sqrt{\text{sample size}}$. The 95% confidence intervals for an individual patient in the study group (mean $\pm$ 2 SD) for the difference between central and mixed venous oxygen saturation are therefore ($Scv_{O_2} - \bar{Sv}_{O_2}$) = –2.57 to 8.77%.

Using the above range of measurements for Scv_{O_2} , the variation in derived oxygen transport variables may be demonstrated by calculating oxygen consumption ($\dot{V}O_2$). The range of $\dot{V}O_2$ values may then be compared against $\dot{V}O_2$ calculated using mean $\bar{Sv}_{O_2}$ (= 70.8%). From the study, mean cardiac index (CI) = 3.3 litre $\text{min}^{-1} \text{m}^{-2}$. If we assume an arterial saturation (Sa_{O_2}) = 97%, haemoglobin (Hb) = 11.5 g dl^{-1} , Hufner factor = oxygen 1.34 ml/g Hb and ignore the small contribution of oxygen dissolved in plasma (< 2% total oxygen content) then we can calculate, using $\bar{Sv}_{O_2}$, that $\dot{V}O_2$ = 133.0 $\text{ml min}^{-1} \text{m}^{-2}$. Using the range of Scv_{O_2} values we can calculate that, for 95% of patients, $\dot{V}O_2$ may vary between 88.4 and 147.2 $\text{ml min}^{-1} \text{m}^{-2}$. We consider this to be a significant potential error in estimation of $\dot{V}O_2$.

We have previously presented data from our unit comparing the differences between pulmonary artery (mixed venous), right atrial and superior vena cava (central venous) oxygen saturations in critically ill patients (mean APACHE II score = 24.6), and showed large variability [4]. Oxygen saturation was measured with an IL 282 Co-oximeter. Blood from the superior vena cava (Ssv_{O_2}), which is the most likely sampling point when using jugular or subclavian vein catheters, showed the widest variation from pulmonary artery saturation (Spa_{O_2}). The mean difference was ($Ssv_{O_2} - Spa_{O_2}$) = 7.93% with a 95% confidence interval for an individual patient of –18.4 to 24.2%. Using these measurements we can calculate $\dot{V}O_2$. The patients reported had mean CI = 4.5 litre $\text{min}^{-1} \text{m}^{-2}$, mean Hb = 11.5 g dl^{-1} and mean Sa_{O_2} = 97%. For comparison with the above, we calculated $\dot{V}O_2$ ignoring the small contribution from dissolved oxygen and used oxygen 1.34 ml/g Hb as the Hufner factor. If blood is taken from the pulmonary artery, mean $\dot{V}O_2$ = 171.4 $\text{ml min}^{-1} \text{m}^{-2}$, while, for blood from the superior vena cava, $\dot{V}O_2$ may vary for 95% of patients between 3.6 and 299.3 $\text{ml min}^{-1} \text{m}^{-2}$.

We conclude that Dr Berridge has shown, in a heterogeneous group of patients, that there are significant differences between central and mixed venous oxygen saturation and has confirmed that blood from central veins cannot be used as a mixed venous sample for calculation of derived oxygen transport variables.

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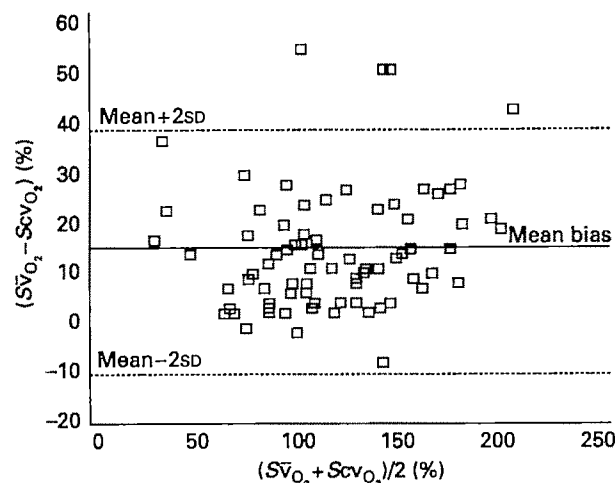


FIG. 1. Bland–Altman plot of two methods of estimating $\dot{V}O_2I$: using $\bar{Sv}_{O_2}$ or Scv_{O_2} .

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Sir,—I suggested, but did not “assert” that, because of a close correlation between central venous and mixed venous oxygen saturations, central venous samples were a useful estimate of mixed venous oxygen saturations. I have already supplied the relevant Bland–Altman data in reply to previous correspondence [1].

Regarding the oxygen transport variables, I have calculated the oxygen consumption index ($\dot{V}O_2I$) for the study population using either mixed venous oxygen saturation or central venous oxygen saturation and the actual haemoglobin concentrations of the patients. The results are as follows: mean $\dot{V}O_2I$ using $\bar{Sv}_{O_2}$ = 126 $\text{ml min}^{-1} \text{m}^{-2}$ (95% confidence intervals 116.6–136.1 $\text{ml min}^{-1} \text{m}^{-2}$); mean $\dot{V}O_2I$ using Scv_{O_2} = mean 113 $\text{ml min}^{-1} \text{m}^{-2}$ (95% confidence intervals 103.8–121.5 $\text{ml min}^{-1} \text{m}^{-2}$). The Bland–Altman plot of the two methods of estimating $\dot{V}O_2I$ is shown in figure 1. Mean bias is 13.7 $\text{ml min}^{-1} \text{m}^{-2}$ and limits of agreement are –11.4 to 38.8 $\text{ml min}^{-1} \text{m}^{-2}$. The usefulness or otherwise of such data is for the individual to judge. I cannot explain the difference between my findings and other authors, except that there was a deliberate attempt to position the central venous catheter optimally, clinically, and that a large proportion of the patients I studied were undergoing cardiac surgery and did not have sepsis.

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1. Berridge JC. Statistical assessment of two methods of clinical measurement. *British Journal of Anaesthesia* 1993; 70: 595.

PIPECURONIUM-INDUCED PROLONGATION OF VECURONIUM BLOCK

Sir,—Drs Smith and White [1] explained the prolongation of vecuronium block after partial recovery from pipecuronium on the basis of residual receptor occupancy by the pipecuronium. They attributed this to a presumed plasma concentration of pipecuronium about 50 min after administration of 10 $\mu\text{g kg}^{-1}$. In our study, we demonstrated prolongation of vecuronium block after 50% recovery from pancuronium and a decrease in duration of pancuronium block after 50% recovery from vecuronium. In these experiments, the influence of the plasma concentration of drug and therefore of any pharmacokinetic influence was obviated

by performing the two experiments simultaneously using the two isolated forearms of volunteers [2]. Thus at the time of the second administration of the drugs the background minimal plasma concentration was the same in both arms.

A further argument against this phenomenon being caused by acetylcholine receptor occupancy is our observation that, at the point of 100% recovery of T1 and presumably about 80% residual receptor occupancy, no effect of the conditioning drug upon the second drug was observed. As a result of this and other observations, we concluded that the effect described was the result of biophase binding in the effect compartment and was not directly dependent upon pharmacokinetic events [3].

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1. Smith I, White PF. Pipecuronium-induced prolongation of vecuronium neuromuscular block. *British Journal of Anaesthesia* 1993; 70: 446-448.
2. Feldman SA, Fauvel NJ, Hood JR. Recovery from pancuronium and vecuronium administered simultaneously in the isolated forearm and the effect on recovery following administration after cross-over of drugs. *Anesthesia and Analgesia* 1993; 76: 92-95.
3. Feldman S. Biophase binding: Its effect on recovery from non-depolarizing neuromuscular block. *Anesthesia Pharmacological Review* 1993; 1: 81-87.

Sir,—We thank Professor Feldman and Dr Hood for drawing our attention to their recent work, which had not been published when our manuscript was submitted to *British Journal of Anaesthesia*. They have confirmed our clinical finding [1] that neuromuscular blockers administered during partial block have their duration of action modified by the residual drug [2]. We agree that this interaction is *not* a result of residual plasma concentrations, and did not intend to suggest such a mechanism in our article.

We referred to the ACh receptor in rather loose terms, but concede that the authors are more correct to suggest that this phenomenon takes place in the "biophase". However, there is no proof of the exact anatomical location at which the biophase interaction occurs.

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1. Smith I, White PF. Pipecuronium-induced prolongation of vecuronium neuromuscular block. *British Journal of Anaesthesia* 1993; 70: 446-448.
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EFFECT OF NITROUS OXIDE ON CEREBRAL BLOOD FLOW

Sir,—We were interested to read the paper by Field and colleagues [1] on the effect of nitrous oxide on cerebral blood flow (CBF) in humans. They interpreted the data to suggest a cerebral vasodilator action of 30% and 60% inspired nitrous oxide compared with pure oxygen.

Before criticizing the use of nitrous oxide in neuroanaesthesia, we wonder if their data may also be interpreted as confirmation of the well-established cerebral vasoconstrictor properties of oxygen. Inhalation of 100% oxygen typically reduces CBF by 10-15% [2, 3]—an order of magnitude similar to that of the change ascribed to nitrous oxide. The design of their experiment was compromised by the fact that, as the subjects inhaled a greater concentration of nitrous oxide, they also breathed a smaller

concentration of oxygen. This can be avoided only by introducing an inert third gas.

Moreover, we argue that it would be more relevant also to clinical practice to maintain the inhaled oxygen concentration constant: the practical choice is really between (say) 30% oxygen and 70% of either nitrous oxide or nitrogen.

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Sir,—Drs Dirnhuber and Davies have suggested that our data may be interpreted as confirmation of the well-established cerebral vasoconstrictor properties of oxygen.

We thank them for the opportunity to challenge the view that inhalation of 100% oxygen typically reduces cerebral blood flow (CBF) by 10-15%. The studies by Kety and Schmidt [1] and Lambertson and colleagues [2] on which this assumption is based were flawed in that the volunteers taking part hyperventilated whilst inhaling 100% oxygen. A small decrease in mean arterial carbon dioxide tension of 0.13 kPa in the former study and a greater change of 0.26 kPa in the latter, whilst not statistically significant, could account for 4% and 8% reductions in CBF, respectively [3]. Lambertson and colleagues pointed out that a 267-kPa increase in arterial oxygen tension together with a 0.67 kPa reduction in arterial carbon dioxide tension do not result in a reduction in CBF greater than that produced by a 0.67-kPa reduction in carbon dioxide tension alone [1]. Furthermore, he concluded that "a physiologically important specific vasoconstrictor action of oxygen probably does not exist in the intact human brain" and that reduced CBF during oxygen inhalation may be explained by a primary hyperventilation and reduction in arterial carbon dioxide tension.

Our volunteers did not hyperventilate as confirmed by a stable end-tidal carbon dioxide concentration throughout the study. We believe that further information is required on the influence of moderate hyperoxia (for instance that produced by inhalation of 40-100% oxygen) on cerebral haemodynamics before the findings of Kety and Lambertson can be validated.

Our purpose was to mimic as closely as possible the clinical situation in which nitrous oxide is administered in varying concentrations with oxygen. The introduction of a third gas in the equation does not conform with current anaesthetic practice. We do not wish to criticize the use of nitrous oxide in neuroanaesthesia, merely to shed some light on the conflicting evidence surrounding its effects on cerebral haemodynamics: we believe, for the reasons explained above, that our data reflect the effect of nitrous oxide on CBF.

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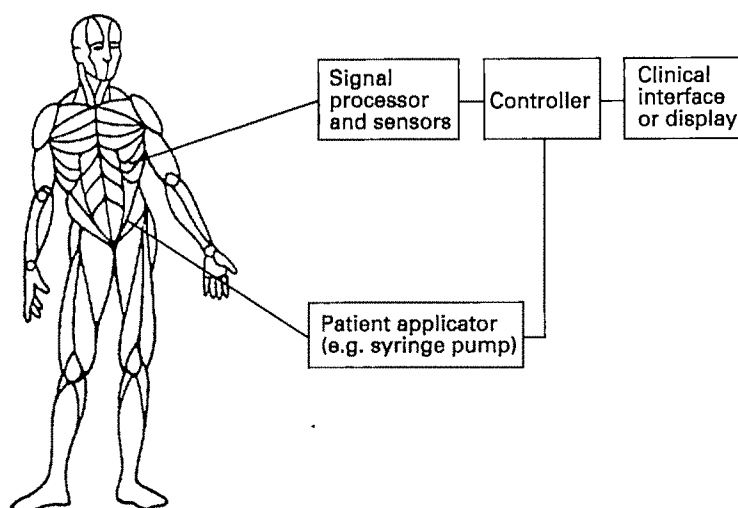


FIG. 1. Block diagram for a closed-loop anaesthesia control system.

SOFTWARE SAFETY CONSIDERATIONS IN THE USE OF CLOSED-LOOP, PATIENT-CONNECTED CONTROL SYSTEMS

Sir,—The recent paper by Chaudhri and colleagues, on the evaluation of a closed-loop device for control of arterial pressure during hypotensive anaesthesia [1], prompts the general question of what principles should be applied to ensure such closed-loop systems are not hazardous.

Any closed loop anaesthesia control system can be represented by a block diagram (fig. 1).

In general, the physiological signal processor is a theatre or ITU monitor of some type. In the study by Chaudhri's group this was a Datascope monitor. Similarly, the patient applicator may be a syringe pump, as used by Chaudhri and colleagues. These are devices sold for medical use and may be expected to comply with a recognized safety standard such as BS5724; they have independent alarms, interlocks, or both.

In fact, all the elements in such a closed loop could be designed and built as electronic modules conforming to BS5724. However, it is more likely that the controller and its interface would be implemented as software in a microcomputer system.

Such closed-loop controllers are clearly safety-critical devices as there exists a potential for malfunctions that can cause the death or serious injury of the patient. The software-comprising part of these systems is therefore a safety-critical software module of the most sensitive grade. Performance in terms of the definitions of software safety ranks with systems to fly aircraft, or those used to control nuclear power stations. For such safety-critical systems, considerable time and effort has been spent by different authorities to lay down guidelines and procedures for the development of safe or reliable software.

Under the present standards, designing safe software of this type starts with a formal specification of the problem, preceded by hazard analysis. When the code is written, some software languages may have to be rejected as being intrinsically less safe than others. In a recent draft of the new IEC guidelines, the language "C" was classified as being unsuitable for safety-critical applications. At the end of writing the code to implement the specification, a formal procedure of checking the software against the specification is recommended. External audits of function by third parties are often deemed necessary. Testing the basis, logic and reliability of the software may go as far as formal mathematical techniques or the use of advanced automated systems for proving the theorems underlying the construction of the code.

The problem of software safety does not, however, stop with well written, validated code. The program must run in a computer. The reliability and safety of the computer may, in some respects, be checked against the specifications such as BS5724, although there are virtually no suppliers of computer equipment that even suggest that their equipment is built to BS5724 standard and suitable for connection to patients. However, even if a computer hardware is reliable, it includes factors such as operating systems which are in themselves computer programs and may have

potentially dangerous flaws in them—for instance, the compiler used to compile the machine code from the source code may be defective. Even if all these elements are without fault in themselves, they may interact in an unexpected fashion to generate a potentially dangerous state.

In the defence industry, the response to this problem has been to certify the use of certain compilers, prohibit the use of certain languages and develop, at great cost, specific computer systems with known safety properties.

The process I have outlined is both complex and expensive. It will be beyond most of the researchers who may write safety-critical software for experiments or experiment with closed-loop anaesthesia systems. The question arises then, what reasonable steps may be expected of workers in this area? In the interests of starting a debate, I suggest the following list:

- (1) The concept of "safe under single fault conditions" embodied in BS5724 be extended to software dependent systems.
- (2) That independent watchdogs, interlocks or alarms for any critical feature be available as an override independent of the computer system used. These interlocks should be in independent systems and should not be software-dependent.
- (3) All safety-critical alarm conditions should be duplicated independently from the software in the system. For instance, the alarm systems of the patient application and signal processor should be set to realistic limits, to provide independent warnings.
- (4) Software should be validated over an acknowledged range of input variables.
- (5) A computer system controlling a safety-critical system should not form part of any local area or other computer network, to reduce the possibility of interaction. It should be the sole occupant of the system upon which it functions. The number of input/output devices required should be minimized.
- (6) The operation of the software should not obliterate or interfere with the normal functioning of the displays or operations of monitoring equipment used in conjunction with the system.

Software-generated accidents are not a myth. They have already claimed lives. The potential for disaster in this area is very great. As a result I wish to suggest one more step.

As the problem of software criticality is known and recognized it would be, in my view, almost certainly negligent for any engineer or clinician to build a system and use it with patients without taking appropriate steps. I should like to propose that, if anaesthetists use critical software in their studies, they be required to show that the procedures they use have been reviewed by a third party and that they conform to the above or a similar standard.

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1. Chaudhri S, Colum JR, Todd JG, Kenny GNC. Evaluation of closed-loop control of arterial pressure during hypotensive anaesthesia for local resection of intraocular melanoma. *British Journal of Anaesthesia* 1992; 69: 607–610.

Sir,—The general points made by Dr Beatty concerning the use of patient-connected, closed-loop control systems are valid in that there is a requirement to undertake hazard and failure analysis and to ensure that the system is as fault-tolerant as possible. However, it is important to realize that, while a closed-loop system for use during anaesthesia is a safety-critical device, the criticality of the software does not rank with that of programs for aircraft control systems and nuclear power control systems, as the system may be overridden by the attendant anaesthetist at any time. In contrast, aviation systems must be fault-tolerant, maintaining stability after failure of the software. A fault in a system designed for anaesthesia can be detected and the system disabled. This is certainly not the case in an inherently unstable aircraft which is unable to fly without computer control. Care should be exercised when making reference to the defence industry, prohibiting certain languages and insisting on the use of validated compilers. The complexity of a multi-computer system monitoring hundreds of moving targets in real time is far more complicated than the clinical system which generated this discussion. The compilers are very expensive and may only run on special microprocessors.

One major point which Dr Beatty has omitted is that it is essential to have an accurate input signal to any closed-loop system. Artefact rejection is therefore of equal importance to the safety of the control software. Similarly, it is vital that the output device has been designed appropriately for the purpose of closed-loop control. The output device must be capable of reliable drug delivery to the patient, reliable control from the processor and highly secure communication between the output device and the processor. Proscription of certain programming languages will not ensure that a system is safe. All languages may be abused and used to write poor software. Many researchers can program in BASIC or C and for this reason alone these languages are suitable for the development of prototype, real-time systems. Hardware safety may require separate control and protection channels in addition to redundancy with a second processor and program which are identical to the first, or diversity where the second processor and program are different and run in parallel with the first. Separation of the control and monitoring functions (both hardware and software) of the system is both easier to demonstrate and more convincing in terms of overall safety.

Software safety is, therefore, only one aspect of the overall safety of any system. Another factor is that closed-loop systems designed for use in anaesthesia are not intended to replace the anaesthetist. They aim to provide an improved technique for drug delivery which is similar to that obtained using an infusion pump compared with a simple i.v. infusion, but the responsibility for controlling the drug remains that of the anaesthetist.

In addition, there are two phases involved in the development of any closed-loop system:

(1) The production of the initial design which is able to undertake the tasks required. This is performed usually in a closely monitored environment such as an operating theatre where the system is monitored closely and frequently by the supervising anaesthetist. The safety aspects of such a system are obviously of a lesser nature than those involved with controlling aircraft or nuclear power stations where there is less opportunity for detailed supervision and intervention by the users.

(2) Subsequent development of this system into a form which can be used routinely by suitably trained personnel and which may then be available for general use.

It is important to distinguish between these two phases, as there is a greater requirement for safeguards with the latter. In designing real-time software for clinical research, it is important that a system hazard analysis be conducted and a specification written for the system. The analysis should show up the potential failures such that the best hardware or software measures may be implemented to prevent the failures leading to an incident. Further, for clinical trial purposes it may be more appropriate to write some manual monitoring of the system into the trial procedure. This option is not open to manufacturers of critical equipment, as they have less control over how the equipment may be used. The production of a system for general use usually involves co-operation with an industrial partner and, at that stage, it is clear that more detailed and complex strategy for development and evaluation must be used. Even when the highest degree of software safety has been implemented, problems remain of adequate clinical testing, as it is difficult to anticipate all effects or to test all possibilities and combinations of events. For this reason, it is essential to use persons other than those who developed the

original system to find unsuspected faults in the overall or detailed design.

These comments must apply, not only to closed-loop control systems, but also to any devices based on microprocessor technology, such as infusion pumps, patient-controlled analgesia systems and ventilators, which are used to deliver critical therapy to patients, because the underlying concerns about hardware and software safety are equally valid. I would suggest that Dr Beatty should provide anaesthetists with the benefit of his expertise by presenting his views and proposals on this matter to the Society for Computing and Technology in Anaesthesia.

G. N. C. KENNY
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Sir,—Thank you very much for the opportunity to contribute to the discussions on software safety considerations in the use of closed-loop, patient-control systems, particularly those prompted by Dr Beatty's comments.

From the practical point of view, automatic control of neuromuscular block, arterial pressure and even adequacy of anaesthesia are very much in their infancy and all such techniques are operated under the close supervision of anaesthetists in theatre. There is, for example, no likelihood that such a system would be set in motion and the anaesthetist would depart.

Dr Beatty's concerns are plainly reasonable, but we have not yet reached the degree of sophistication that would allow these to be implemented and certainly it is highly unlikely that feedback control systems will ever be allowed to work without direct supervision in theatre. As feedback control systems develop, they will obviously have to include many of the features that Dr Beatty has outlined, such as watchdog facilities, and some already do.

In addition, one of the major concerns about medical data which may even prevent feedback control systems ever "taking over" is the problem of artefacts. Before feedback control systems can even be designed with the sophistication suggested by Dr Beatty we need to become much improved at recognizing and rejecting data containing artefacts.

It is also important when developing new methods of administering drugs to ensure that an Ethics Committee gives approval having reasonably understood the additional risks involved. Subsequently, a patient who gives approval for his anaesthetic to be conducted by a feedback control mechanism should also give informed consent and should understand that the method of controlling the drug dosage is experimental, but that it could revert to a normal method within seconds. The patient must have some comprehension of the additional risks posed by the use of automatic control techniques.

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A SIMPLE ASPIRATION TEST CAN DETECT TOURNIQUET FAILURE DURING I.V. REGIONAL ANAESTHESIA

Sir,—The recent article by Rawal and colleagues [1] was remarkably complete in its discussion of modification of the technique of i.v. regional anaesthesia, in particular the re-exsanguination of the participating hand shortly before skin incision to remove excess local anaesthetic and therefore provide a dry operating field.

Unfortunately, we have no control over the non-compressible intramedullary vessels so that correct management of the tourniquet after adequate exsanguination, such as the position, width and the pressure of the applied cuff, is still crucial for the successful block.

We have used a simple aspiration test which, in our opinion, may be applied to differentiate primary tourniquet failure from other possible causes of venous distention.

Plourde and colleagues in their angiographic study [2] found that the volume of the forearm venous system in a 70-kg man is 17.7 (SD 4.7) ml; thus it seems logical to speculate that a significant portion is removed by routine exsanguination. Therefore, a positive aspiration test (blood-tinged fluid) conducted at the time of slow i.v. injection of the initial portion (5–8 ml) of local anaesthetic is highly suggestive of cuff incompetence and should be treated as such by cessation of the injection, reapplication of the Esmarch bandage and recommencing the entire procedure.

For the past 3 years this technique has been used successfully in this institution on 128 occasions; tourniquet inadequacy was detected in 11 instances (8.6%).

I realize that the proposed aspiration test is not a foolproof criterion of tourniquet failure, but rather an additional bed-side tool which may contribute greatly to patient safety. I recommend it for routine use in i.v. regional anaesthesia.

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CENTRAL VENOUS CANNULATION IN PAEDIATRIC PATIENTS

Sir,—In their paper describing the use of ultrasound to facilitate central venous cannulation in paediatric patients, Dr Alderson and colleagues [1] found that a frequent problem was inability to pass the guidewire through the introducer needle and onwards into the superior vena cava, particularly when performing the technique in neonates and infants. It was postulated that this may result from the ease with which the tip of the introducer needle becomes displaced from the lumen of the vein as the aspirating syringe is disconnected from the needle.

Although the authors stated that this problem has not been reported in other studies, it is certainly not uncommon in clinical practice. It may, however, be eliminated by the prior insertion of a standard i.v. cannula into the internal jugular vein: the full length of the cannula is advanced into the vein and a syringe attached to confirm correct placement; the guidewire may then be passed through the cannula into the vein. The cannula is removed and the central venous device inserted over the guidewire in the usual way. It is clearly important to ensure that the selected guidewire will pass through the cannula which has been selected. In neonates and small infants, I usually use a Cook 4-French gauge double-lumen device; the guidewire with which it is provided will pass through the lumen of a standard 22-gauge cannula. I am sure that I am not alone in using this simple modification which allows the guidewire to be passed with ease and is associated with few failures, even in small children.

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1. Alderson PJ, Burrows FA, Stemp LA, Holtby HM. Use of ultrasound to evaluate internal jugular vein anatomy and to facilitate central venous cannulation in paediatric patients. *British Journal of Anaesthesia* 1993; 70: 145-148.

Sir,—Although the technique Dr Bennett describes is certainly an excellent alternative to the Seldinger technique, we used the Seldinger technique in our study for two reasons; first, it is the preferred technique at the Hospital for Sick Children (Toronto) and second, it is the standard technique described for insertion of jugular venous lines.

In our institution, we have found greater success in locating the internal jugular vein (IJV) and passing the guidewire using the Seldinger technique than by using the i.v. catheter technique described by Dr Bennett. We agree that when cannulated successfully, the i.v. catheter technique greatly facilitates the passage of the guidewire. However, we have found considerable

difficulty in successfully passing the i.v. catheter into the IJV. This difficulty most likely reflects our lesser experience with this particular technique.

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EFFICACY OF CONTINUOUS INTERCOSTAL BUPIVACAINE FOR PAIN RELIEF AFTER THORACOTOMY

Sir,—We wish to comment on the technique described recently by Dryden, McMenemin and Duthie [1]. We disagree with the comment "it should not be assumed that an intercostal injection of contrast medium and an intercostal infusion of bupivacaine behave similarly".

We have demonstrated previously, by infusing methylene blue peroperatively [2] and by contrast computed tomography on the 5th day [3] of the infusion, that the site of action of continuous extrapleural intercostal nerve block was in the paravertebral space. Using a single catheter, the contrast medium was confirmed to be in the paravertebral space covering, on average, six intercostal spaces. The unilateral sympathetic block that accompanies this technique may result in transient Horner's syndrome, particularly with high thoracotomy incisions [3]. We used simultaneous bilateral optical plethysmography [4] over the appropriate dermatomes, to demonstrate complete unilateral sympathetic block.

Paravertebral block affects not only the intercostal nerves, which in this area are not invested with fascial sheath, but also the sympathetic chain and the posterior primary rami which mediate pain from straining of the ligaments of the costovertebral and costotransverse joints and the posterior spinal muscles [2]. The sympathetic nervous system is involved in each stage of the nociceptive response to acute injury, such as that associated with surgery [5]. Evidence suggests that the peripheral afferent block is more effective in preventing nociceptive impulses entering the central nervous system than the central block, as paravertebral block would also reduce neurogenic inflammation of traumatized tissues that is dependent on efferent functions of peripheral nerves [6, 7].

The exact distance of the tip of the catheter from the vertebral bodies has not been stated in the report; according to their description and the accompanying illustration, it appears too lateral to block effectively the sympathetic chain and the dorsal primary rami in addition to a standard intercostal nerve block. This explains their less than ideal result. Placing the catheter in the paravertebral space [2] obviates the need for more than one catheter, as the spread of local anaesthetic agent averaged six intercostal spaces [2, 3].

S. SABANATHAN
J. RICHARDSON
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1. Dryden CM, McMenemin I, Duthie DJR. Efficacy of continuous intercostal bupivacaine for pain relief after thoracotomy. *British Journal of Anaesthesia* 1993; 70: 508-510.
2. Sabanathan S, Mearns AJ, Bickford-Smith PJ. Efficacy of continuous extrapleural intercostal nerve block on post-thoracotomy pain and pulmonary mechanics. *British Journal of Surgery* 1990; 77: 221-225.
3. Eng J, Sabanathan S. Site of action of continuous extrapleural intercostal nerve block. *Annals of Thoracic Surgery* 1991; 51: 387-389.
4. Kamal AAR, Harnes JB, Irving G, Mearns AJ. Skin photoplethysmography—a review. *Computer Methods and Programs in Biomedicine* 1989; 28: 257-269.
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7. Coderre TJ, Katz J, Vaccarino AL, Melzack R. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain* 1993; 52: 259-285.

Sir,—I agree entirely with this view that paravertebral block would provide reduction in requirement for morphine, greater than that achieved with the intercostal block used in our technique.

Several of the chest radiographs, including the one in the article, demonstrated contrast medium in the paravertebral space. The statement "it should not be assumed that an intercostal injection of contrast medium and intercostal infusion of bupivacaine behave similarly", referred only to our study and was intended as a suggestion as to why we had a paravertebral injection radiographically, but an intercostal injection clinically.

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WHOLE BOWEL IRRIGATION IN ACUTE POISONING

Sir,—We read with interest the review on management of acute poisoning by Collee and Hanson [1]. We suggest an additional intervention which may occasionally be useful in reducing absorption of some poisons from the intestine. There are situations in which induction of vomiting, gastric lavage and administration of activated charcoal may be inadequate. These include: late presentation after ingestion when significant amounts of drug have entered the small intestine; ingestion of very large amounts when the residual drug remaining after gastric emptying may still be present in toxic amounts; ingestion of a drug, such as iron, which is not adsorbed by activated charcoal.

Under these circumstances a useful treatment is the use of whole bowel irrigation. This consists of rapid enteral administration of large amounts of lavage fluid over several hours as in preparation for colonoscopy or large bowel surgery.

We have recently treated six children aged from 18 months to 5 yr who presented after ingestion of the tricyclic antidepressant dothiepin (Prothiaden). There was uncertainty about the amount ingested and the interval between ingestion and presentation, although in no case was this interval less than 1 h. In four of the patients, there were symptoms which suggested that the drug had passed into the small intestine from where it was being absorbed. Four patients were given ipecacuanha to induce vomiting and two underwent gastric lavage. Because of concern that further absorption of the drug would occur from the small intestine, these six children underwent whole bowel irrigation.

This was administered as polyethylene glycol sulphate via a nasogastric tube at 500 ml h⁻¹ and continued for 6 h or until the rectal effluent was clear. Plasma electrolyte concentrations were measured before, during and after treatment and there were no changes. No patient deteriorated during treatment and those with symptoms improved.

Tenenbein [2] used whole bowel irrigation in eight children after toxic drug ingestion and found it useful in four. There were no changes in electrolyte concentrations or PCV. In a study of healthy volunteers given a large overdose of ampicillin, Tenenbein, Cohen and Sitar [3] found in 67% reduction in drug absorption after whole bowel irrigation. This was more effective than gastric lavage, ipecacuanha-induced emesis and activated charcoal administration which reduced absorption of ampicillin by 32%, 38% and 57%, respectively [4]. The solution we used (polyethylene glycol) is associated with insignificant absorption or secretion of water and electrolytes during lavage [5].

We suggest that this technique may be useful in some cases of acute poisoning.

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C. BEST
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5. Davis GR, Santa Ana CA, Morawski SG, Fordtran JS. Development of a lavage solution associated with minimal water and electrolyte absorption or secretion. *Gastroenterology* 1980; 78: 991–995.

Sir,—We would agree that this technique could be a valuable additional intervention under the following circumstances:

- (1) that there has been a serious intoxication with potential loss of life;
- (2) that the manoeuvre is performed under strict metabolic fluid balance control;
- (3) that there is full control of the airway and, where it is compromised, intubation is performed before irrigation. Whole bowel irrigation should be conducted, if at all possible, in an Intensive Care Unit.

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G. COLLEE
Royal Free Hospital
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BOOK REVIEWS

Case Presentations in Anaesthesia and Intensive Care. By K. J. Power. Published by Butterworth-Heinemann, Oxford. Pp. 127; indexed; illustrated. £12.95.

This little book consists of 31 presentations of cases requiring anaesthesia or intensive care. The cases include all the types which may be seen in the F.R.C.A. examination, but which appear rarely in clinical practice—for example porphyria, acromegaly, malignant hyperthermia, pulmonary hypertension—in addition to more everyday conditions such as severe ischaemic heart disease, asthma and emphysema. Several clinical situations, such as the trauma patient, the drug overdose case and sudden intraoperative hypoxaemia, are also considered.

For each case presentation there is a brief but pertinent description of history and clinical examination followed by relevant investigations, including illustrations of chest x-rays or ECG. Thereafter, a number of questions are posed, followed by a discussion of the case which culminates in recommendations for anaesthetic management.

There is always a tendency for a book such as this to include "classic" cases whereas, in the real world, cases are less black and white; to some extent that is true here. The facts given are inevitably slanted towards a certain diagnosis. However, in the clinical situations included, the cases are less clear cut and the discussion does manage to mimic the real situation.

I thoroughly enjoyed reading this book, and would imagine that a trainee revising for the F.R.C.A. would find it a very useful break from the drudgery of normal textbooks. It might be particularly helpful during the period between written and oral examinations when conventional revision often proves difficult.

The author has exposed to potential criticism his opinions regarding the management of a whole range of different conditions and situations, and inevitably one can quibble with some of his recommendations—especially, perhaps, in the intensive care/emergency/trauma situation in which management options are less clear cut. For example, case presentation No. 3, a high medical risk elective aortic aneurysm case, should never have been in a high dependency unit after operation, but rather in an intensive care unit. His renal failure should have been noted before 48 h, and a pulmonary artery catheter inserted to measure cardiac output and oxygen delivery and to guide appropriate cardiovascular therapy. Instead, the acute renal failure is just accepted, and all the discussion revolves around the subject of haemofiltration. "Renal" dopamine is also accepted without question, when in fact it is of no proven worth.

One may also take issue with the sequence of events in the management of the chest trauma (presentation No. 8). In this situation, one must not fail to mention administration of high flow oxygen; also there was need for action on three fronts virtually simultaneously: intercostal drainage, tracheal intubation and i.v. cannulation. Urgent intercostal insertion of a large-gauge cannula was indicated before formal drain insertion.

Overall, however, the author argues his way through his presentations well, imparts useful information to the reader and provides a helpful new slant to the candidate preparing for the Fellowship's examination.

I. S. Grant

The History of Anaesthesia: Third International Symposium Proceedings. Edited by B. R. Fink, L. Morris and R. Stevens. Published (1992) by Wood Library-Museum of Anesthesiology, Park Ridge, Illinois. Pp. 467; indexed; illustrated. Price (paperback) U.S. \$70; (hardback) U.S. \$80.

This book is the third in a series of International History of Anaesthesia Symposia Proceedings and is based on the meeting held in Atlanta, Georgia in late March 1992, which commemorated the 150th Anniversary of the use of ether by Crawford Long in nearby Jefferson, Georgia.

This volume differs in size and presentation from the two previous History Symposia Proceedings published to date. In their desire to produce this book with the minimum of delay, the Editors confess to an acceptance of a variety of written styles which, in this reviewer's opinion, does not detract from the volume in any respect. The three co-editors for this book are renowned anaesthetists and anaesthetic historians. As would be expected from such a triumvirate, their efforts are excellent and the resulting book is a real pleasure to read.

The papers are published in alphabetical order of first author, but may also be accessed rapidly through the several indexes provided which group papers under the broad anaesthetic categories of apparatus, agents and techniques; biography; military; and general. There is a further alphabetical index of topics, and a complete author index. This styling allows readers to find their way round the book with ease. The page layout is simple and the typesetting is in a font size which is "user-friendly" for the older reader.

More than 90 papers are published; some are only abstracts of up to one page in length, while others are small booklets in their own right. The majority of papers are enhanced by illustrations, some of which appear in colour. The extensive lists of references associated with the papers make the volume worth buying for this reason alone.

The book clearly reflects the international flavour of the meeting, with contributions from six continents and 16 countries. Predominant amongst these are papers from the U.S.; these constitute 55% of the book, which makes the volume of great interest to the European market, which is rarely able to read such contributions. It is invidious to pick out single authors, but Michael Goerig from Hamburg is noteworthy for having his name associated with eight papers. The International Historical world of anaesthesia is obviously in agreement, as Dr Goerig will be hosting the Fourth International Symposium on the History of Anaesthesia in 1997 in Hamburg.

Everyone with a major interest in the history of anaesthesia will want their own copy of this excellent book, but it should be in every Departmental library where it will provide considerable interest and pleasure to any anaesthetist who "dips" into its pages. Anyone who doubts this last statement should reflect on the words of George Santayana, "those who ignore the lessons of history, shall be forced to repeat them."

D. J. Wilkinson

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